1-1-2000

RAPD-derived markers linked to the Mla resistance gene cluster in barley

Karin Sue Gobelman-Werner
Iowa State University

RAPD-derived markers linked to the *Mla* resistance gene cluster in barley

by

Karin Sue Gobelman-Werner

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Plant Pathology

Major Professor: Roger P. Wise

Iowa State University

Ames, Iowa

2000

Graduate College
Iowa State University

This is to certify that the Master's thesis of

Karin Sue Gobelman-Werner

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

This thesis is dedicated to my family, who has suffered through the education process with me.

To my husband, Larry, who learned to tell time the laboratory way, i.e. one hour of laboratory time is always equal to or greater than four hours of normal time.

To my children, Larry (Buck), Barret (Bear) and Logan (Angus), who think it is neat that mom went back to school and will be glad when the degree is accomplished.

I thank them all for their patience and love.

TABLE OF CONTENTS

RAPD-Derived Markers Linked to the *Mla* Resistance Gene Cluster in Barley

Karin Sue Gobelman-Werner

Major Professor: Roger P. Wise
Iowa State University

Powdery mildew of barley, caused by *Erysiphe graminis* f. sp. *hordei*, is an obligate fungal pathogen that is distributed widely throughout the world. *Ml* genes mediate resistance to *E. graminis* in barley. The *Mla6, Mla14, Mla13,* and *Ml-Ru3* variants are clustered together and located on chromosome 5 (1H).

Three markers linked to the *Mla* locus were identified using random amplified polymorphic DNAs (RAPDs) and bulked segregant analysis. Seven hundred and thirty-nine RAPD primers were used to screen pools containing C.I. 16151 (which contains the *Mla6 + Mla14* resistance specificities) and C. I. 16155 (which contains the *Mla13 + Ml-Ru3* resistance specificities). A 1500-bp DNA fragment amplified by primer OPA-10 mapped between *Hor1* and *XChs3*. Similarly, a 950-bp DNA fragment amplified by primer UBC-465 is located between *XChs3* and *Xmwg068*. Finally, a 1626-bp DNA fragment amplified by primer UBC-165 mapped 0.28 cM proximal to the *Mla* cluster. Twenty sequenced-tagged site (STS) primers were developed from the *UBC-1652*$_{1626}$.sequence. Eight STS products from *UBC-1652*$_{1626}$ primers mapped 0.28 cM distal to the *Mla* resistance gene cluster. The *FR1062* marker primer pairs P0 and P1034RC were used in the identification of tightly linked YAC clones from the cultivars Ingrid and Franka.

GENERAL INTRODUCTION

Thesis Organization

This thesis describes the identification of RAPD-derived markers in barley. The literature review describes of the relationship between a plant host and fungal pathogen, genetic maps and the techniques involved in developing new markers. References are listed following both the literature review and the paper. The paper "RAPD-Derived Markers Linked to the *Mla* Resistance Gene Cluster in Barley " is to be submitted electronically to the Journal of Agricultural Genomics (JAG). A general conclusion follows the paper. Additional information is included in three appendices; the first appendix is the original RAPD data, the second appendix is the preliminary research for setting up RAPDs and bulked segregant analysis and the third appendix has additional references for trouble-shooting PCR.

Literature Review

Barley

Barley (*Hordeum vulgare* L.) is one the oldest cultivated crops and the fourth in hectares harvested after wheat, rice, and maize (Briggs 1978; FAOSTAT Database, 1998, http://www.apps.fao.org: 8080/servlet/XteServlet?Areas=862& Items=1817&). This cereal crop is primarily grown in northwestern Europe, a few selected areas in North and South America, Australia and Asia. Barley is a true diploid with a haploid chromosome number of seven and a large genome of approximately 5,360-Mb (Bennett and Smith 1976; Arumuganathan and Earle 1991). Contemporary breeding practices have improved barley yield, grain quality, kernel weight and many other agronomic traits. However, when grown in a cool, wet climate, barley can be highly susceptible to powdery mildew, which is caused

by the obligate fungal pathogen, *Erysiphe graminis* DC. Merat f. sp. *hordei* Em. Marchal.

Powdery Mildew Resistance

When inoculated by various races of *E. graminis*, different barley cultivars may express unique infection types to the pathogen, thus allowing the identification of individual resistance genes (Wise and Ellingboe 1983). Studies of the reaction of barley lines to various pathogens indicate that cultivars can possess more than one resistance gene (Wiberg 1974). Most of the genes that confer resistance to powdery mildew in barley (designated *Ml*) are located on chromosomes 2, 4, 5, and 6. These genes are assigned to 11 distinct groups: *MlLa* lies on chromosome 2 (2H) (Giese *et al.* 1993); *Mlg* and *mlo*, are positioned on chromosome 4 (4H) (Hinze *et al.* 1991; Görg *et al.* 1993); *Mlat*, *Mla*, *Mlk*, *Mlnn*, *Mlra, MlGa,* and *Mlp* are located on the short arm of chromosome 5 (1H) (reviewed by Jørgensen 1994); and *Mlh* lies on chromosome 6 (6H) (Søgaard and Wettstein-Knowles 1987; Jørgensen and Jensen 1976). At least 30 *Mla* specificities in barley have been identified which confer resistance to *E. graminis* (Jørgensen and Moseman 1972; Jørgensen and Jensen 1976; Hinze *et al.* 1991; Görg *et al.* 1993; Jørgensen 1994; Kintzios *et al.* 1995). The relationship between the avirulence genes of the pathogen and the resistance genes in the host creates a platform for host-pathogen interaction in barley to be studied.

Due to the susceptibility of barley to a variety of diseases, many of its agronomic traits are affected. The identification and incorporation of new resistance genes into otherwise susceptible barley cultivars would be of considerable agronomic and commercial importance. However, traditional breeding strategies aimed at introducing resistance genes into susceptible cultivars tend to be time consuming, costly and often result in undesirable linked

genes also being introduced unintentionally. New technologies are now making advancements in manipulating the genetic makeup of the cultivated barley (Cocking 1990). Indeed, one of the major current goals in plant biological research is the clarification of the complex relationship that exists between host and pathogen at the molecular level.

Many plant-pathogen interactions demonstrate a gene-for-gene relationship. Flor (1956) first defined the gene-for-gene relationship between the host and pathogen. The resistance gene in the host is not effective unless there is a complimentary avirulence gene in the pathogen. However, this definition only describes the relationship in which major genes determine resistance. Flor (1971) re-adjusted his gene-for-gene relationship definition to account for other genes that may also influence the relationship between the host and the pathogen. For example, the *Mla12* resistance gene confers a race-specific response against the A6 isolate of powdery mildew. Nevertheless, this response also requires both genes *Rar-1* and *Rar-2* to be effective (Freialdenhoven *et al.* 1994). Similarly, Freialdenhoven *et al.* (1996) demonstrated that the effectiveness of the *mlo* resistance gene requires the action of two supplementary genes, *Ror1* and *Ror2*. Hence, resistance genes do not function independently, but rather require other genes. In the development of broad-based resistance, the function of all types of genes must be considered.

Resistance Dependability

Flor (1971) believed that broad-based resistance to a pathogen did not seem possible because of the overloading of the plants with resistance genes. When a resistance gene was introduced into a susceptible cultivar, it sometimes was unstable. The fluctuating resistance to new or different pathogen isolates was

not expected (Wiberg 1974). It has been demonstrated that the intra-specific transfer of traits of agronomic importance such as resistance genes is feasible (Pickering *et al.* 1987; Xu and Snape 1989; Lehmann 1991). However, a simple breeding program using an inter-specific cross of most Hordeum species has been difficult to achieve.

Wild (*Hordeum bulbosum* and *H. spontaneum*) and cultivated barley (*H. vulgare*) are distantly related, but cultivated barley has sensitivity to genetic imbalance (Bothmer *et al.* 1991). Pickering *et al.* (1987) first reported an example of a powdery mildew-resistance gene was transferred from *H. bulbosum* into 81882/83 (*H. vulgare*). The chromosomal position of this gene was determined by restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) analysis and was shown to be located on chromosome 2 (2A) (Pickering *et al.* 1995). The transfer of *H. bulbosum* resistance into cultivated barley is promising since a single gene controls most of the powdery mildew resistance. *H. bulbosum* L. shows resistance to many powdery mildew isolates and has many useful agronomic traits which makes it a likely candidate to be used in a breeding program (Xu and Kasha 1992).

A powdery mildew resistance gene from *H. bulbosum* tetraploid hybrid was transferred into cultivated barley *H. vulgare* in 1992 (Xu and Kasha 1992). Four different *H. vulgare* accessions were crossed by *H. bulbosum*. The progenies were tested with a mixture of powdery mildew isolates to verify presence and stability of the transferred resistance. The transferred *H. bulbosum* DNA was identified using *H. bulbosum* species-specific DNA probes. Further independent testing showed that the transfer of disease resistance and *H. bulbosum* germplasm to be from a single locus.

Once resistance genes are transferred from resistant to susceptible plants, the question of whether or not the various genes were linked to each other needs to be confirmed. Many researchers attempted to verify the transfer of resistance to susceptible cultivars and their linkage to each other. While, Michel (1995) identified a tetraploid-hybrid barley line (14/1/30), which contained *H. bulbosum* DNA. Michel believed that this barley line resistance to powdery mildew was due to a single dominant gene. Pickering (1995) concluded that two different powdery mildew resistance genes that were transferred from *H. bulbosum* into 14/1/30 by Michel (1995) and 81882/83 by Pickering (1987) were closely linked. The powdery mildew resistance genes transferred from *H. bulbosum* by Xu and Kasha (1992) were non-allelic to the powdery resistance gene by Pickering (1987). The confirmation of the linkage between these transferred genes was made easier with genetic maps.

Genetic Maps

Genetic maps are established by the statistical analysis of recombination frequencies, with the frequency increasing with map distance. The map distances are expressed as centiMorgans (cM). Monitoring the segregation of homologous sequences at the DNA level makes possible the creation of a linkage map with a higher saturation of markers. This necessitates the identification of F_2 individuals that carry recombination breakpoints that are located close to a gene of interest. Because breakpoints occur randomly, the probability of a breakpoint lying close to a gene of interest increases as population size increases. Molecular marker positions can then be estimated based on the number of breakpoints occurring between flanking markers.

Detailed maps of the barley genome help in the identification of genes of interest and facilitates the cloning of a particular gene by chromosome walking or landing. Shin *et al.* (1990) constructed a partial map from a cross of multiple recessive marker stock (MMS) and parental two-row cultivar Apex. Graner *et al.* (1991) constructed a genetic map comprised of RFLP markers using two barley populations. The first population consisted of an intra-specific cross of Igri (containing the *Mla8* specificity) and Franka (containing the *Mla6* specificity). The second population contained inter-specific progeny from a cross of Vada x *Hordeum spontaneum*. Heun *et al.* (1991) constructed a map from a cross of the parental cultivars Proctor x Nudinka (containing the *Mla12* specificity). Kleinhofs *et al.* (1993) constructed a map from a cross of the parental cultivars Steptoe x Morex. The Steptoe x Morex map was compared to the MMS x Apex (Shin *et al.* 1990), Proctor x Nudinka (Heun *et al.* 1991), and Igri x Franka (Graner *et al.* 1991) RFLP maps. There was no significant difference in the number of markers placed or the genetic distances on each chromosome among these genetic maps. These RFLP genetic maps exhibit the distance between available markers; however, there are not enough markers to facilitate chromosome landing to any gene of interest.

A high-resolution map facilitates positional cloning of a gene of interest, allows accurate positioning of markers, and resolves tightly linked genes. Frequently, genes conferring resistance are clustered together in the genome, such as the *Mla* locus in barley (Wise and Ellingboe 1985; Jørgensen 1992; Görg *et al.* 1993; Mahadevappa *et al.* 1994; DeScenzo *et al.* 1994), the *Dm* loci in lettuce (*Lactuca sativa* L.) (Hulbert and Michelmore 1985; Farrara *et al.* 1987; Paran *et al.* 1991; Bonnier *et al.* 1994; Maisonneuve *et al.* 1994), the*Rp1* loci in maize (*Zea mayes*) (Saxena and Hooker 1968; Pryor 1987;Hulbert and Bennetzen 1991;

Sudupak *et al.* 1993), and the *Pc* loci in oat (*Avena*) (Rayapati *et al.* 1994; Wise *et al.* 1996).

RFLPs are used to create linkage maps, but are time consuming and generally require the use of radioactive isotopes. These are a few of the implications associated with genetic maps generated by RFLP. New techniques are needed which are quick, easy, non-radioactive and capable of developing more markers located on present RFLP maps.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a technique to amplify small amount of DNA or RNA to high copy number. The PCR process has the advantage of being simple, fast, sensitive, specific, cost efficient and versatile. The disadvantages are the prior knowledge of the sequence required, DNA contamination and the error rate in the Taq polymerase. A basic PCR amplification reaction (Innis *et al.* 1990) depends on the mixture of template DNA (105 to 106 target molecules), oligonucleotide primer, master mix of buffers, deoxynucleotide triphosphates, Taq polymerase, and a thermocycler. A multi-step process of 25 to 35 cycles is performed where different temperatures and time are needed to denature the DNA, anneal the primer, and extend the target DNA. The versatility is in the manipulation of any step of this multi-step process to optimize the protocol for any given PCR application.

Optimization of the following conditions and components eliminates spurious results and can change the result of the experiment. High primer concentration promotes the mispriming and accumulation of nonspecific product that may increase the probability of generating a template-independent artifact (primer-dimer). The magnesium concentration has an extensive affect on primer

annealing, strand dissociation temperature, formation of primer-dimer artifacts, enzyme activity, and fidelity. All four of the deoxynucleotide triphosphates should be used at the same concentrations; low deoxynucleotide triphosphate concentrations minimize mispriming at non-target sites and reduce the likelihood of mis-incorporation of nucleotides. If the concentration of the Taq polymerase is too high, and then nonspecific background products may accumulate; if the concentration is too low, then an insufficient amount of product is made. A denaturation temperature that is too high leads to unnecessary loss of enzyme activity, but a low denaturation temperature results in incomplete denaturation, which allows the DNA strands to "snap back" and thus reduces product yield. Primer annealing temperature and time depends upon the base composition, length, and concentration of the amplification primers. An increase of the annealing temperature enhances discrimination against incorrectly annealed primers and reduces mis-extension of incorrect nucleotides at the 3' end of primers. Primer extension time and temperature depends upon the length and concentration of the target sequence. Too many cycles can increase the amount and complexity of nonspecific background products while too few cycles give low product yield (Innis *et al.* 1990).

A quick, efficient PCR non-radioactive DNA fingerprinting tool that shares the advantages of RFLP without its disadvantages is Random Amplified Polymorphic DNAs (RAPDs). Two different researchers developed the original PCR based fingerprinting technique that required no prior knowledge of the target DNA sequence. The Arbitrary Primed Polymerase Chain Reaction (AP-PCR) developed by Welsh *et al.* (1992) uses a single, arbitrarily chosen PCR primer of 20 to 30 base oligonucleotides to reveal polymorphism between two genomes. Alternatively, Williams *et al.* (1990) established the Random Amplified Polymorphic DNA (RAPD)

technique that detects polymorphism using a single 10 base random oligonucleotides as the primer. Both assays share the advantages of RFLP without the disadvantages. These assays are non-radioactive, fast, simple, applicable to a broad range of species, require only nanogram quantities of DNA, and can produce, in some cases, a dominant marker. However, both techniques were devised at approximately the same time, but it is the RAPD technique of Williams, which is more readily known.

AP-PCR and RAPD techniques are an efficient assay for polymorphism in the rapid identification and isolation of chromosome-specific DNA fragments. These techniques are a link between the genetic and physical maps and are now widely used in breeding programs, genetic mapping, population genetics, and epidemiology. A large variety of organisms including insects, plants, pathogens and many other species have used AP-PCR and RAPD techniques. Examples of some of the species are aphids (Black, *et al.* 1992), grasshoppers (Chapco*et al.* 1992), honeybee (Hunt and P. 1992), apples (Koller *et al.* 1993), rice (Yu and Nguyen 1994), sorghum (Vierling *et al.* 1994), barley (Tinker *et al.* 1993), Norway spruce (Binelli and Bucci 1994), *Brassica* (Demeke *et al.* 1992; Horn and Rafalski 1992; Santos *et al.* 1994), and *Colletotrichum* (Guthrie *et al.* 1992). Questions arose regarding the reproducibility and the mode of inheritance of RAPD markers. The question as to the authenticity of the results shadows the universality and advantages of RAPDs. Studies were done concerning the reproducibility of the RAPD bands between different labs (Penner *et al.* 1993), scoring errors (Skroch and Nienhuis 1995), and inheritance of RAPD markers (Heun and Helentjaris 1993).

Penner *et al.* (1993) concluded if the running conditions are similar, including the type of thermocycler, annealing temperature and especially the

temperature profiles inside the tubes, then reproducibility is achievable. Scoring errors can account for the variation in estimates of genetic distance across independent sets of RAPD bands (Skroch and Nienhuis 1995). The probability of a particular band being consistently scored in duplicate data is dependent on the uniformity of the amplification conditions in the experiment and the relative strength of the RAPD band. Therefore, it is necessary to minimize the differences in the reaction conditions between data generated in repeated experiments. Another problem encountered with the RAPD technique is the complexity involved in the monitoring the inheritance of these markers. RAPDs do not follow the simple dominant inheritance of Mendelian genetics (Heun and Helentjaris 1993). For any particular generated band, inheritance must be verified before the RAPD data can be used with some confidence. Despite the questions about RAPDs, this technique has generated genetic information for a large variety of flora and fauna in a shorter period of time than any other technique.

Another tool to facilitate and expedite the mapping process is bulked segregant analysis (BSA), which involves the pooling of DNA from individuals in a segregating population. One of the advantages of pooling the DNA samples is the ability to target discrete intervals in the genome. Each pool consists of individuals that are identical for the trait or gene of interest but are arbitrary for all other genes (Giovannoni *et al.* 1991; Michelmore *et al.* 1991). Giovannoni *et al.* (1991) uses the pooling of homozygous individuals across a target interval based on known markers within the interval. Alternatively, Michelmore *et al.* (1991) pooled the individuals based on the homogeneity at a single genetic point in a population segregating for the target of interest. Giovannoni's strategy is more versatile and has achieved a greater saturation of molecular markers while Michelmore's strategy is useful for the markers linked to a gene of interest.

Bulked segregant analysis overcomes the problem of trying to isolate new markers near an area of interest when near isogenic lines (NILs) are not available. Nevertheless, optimization of the correct pool size is essential to this technique. If the pool size is too large or small then false positives are generated, but these can be detected with analysis of the segregating population. Pooled individuals define the genetic window surrounding the area of interest by genotype (Reiter *et al.* 1992; Chaparro *et al.* 1994), or by phenotype (Lelner *et al.* 1995). Bulked segregant analysis has been used in the identification of new markers, efficiently filling of gaps in genetic maps or at ends of linkage groups, detecting non-segregating genetic loci, and helping score chromosomal structural and functional loci in a RFLP map. This technique has been used in finding the location of quantitative trait loci (Chalmers *et al.* 1993; Wang and Paterson 1994), the *Rfo* fertility restorer gene in *Brassica* (Delourme *et al.* 1994), and resistance genes in various crop plants (Haley *et al.* 1993; Miklas *et al.* 1993; Mohan *et al.* 1994; Oh *et al.* 1994; Johnson *et al.* 1995; Lelner *et al.* 1995; Mouzeyar *et al.* 1995; Poulsen *et al.* 1995; Wechter *et al.* 1995).

After ascertaining the proper techniques, the products obtained from the combination of the RAPD and BSA techniques may or may not be useful as molecular markers. If the amplified DNA product is not highly repetitive, then the product can be used directly as a RFLP marker. If the amplified DNA product is highly repetitive then it may be necessary to characterize the amplified product in which non-repetitive polymorphic regions can be identified and isolated. Sequenced-tagged site (STS) and sequenced characterized amplified regions (SCARs) are two different techniques used to transform the DNA sequence. Though similar in the design, there are some differences between STS and SCARs.

A sequenced-tagged site (STS) is a short, unique sequence, amplified by PCR, which identifies a known location on a chromosome (Tragoonrung *et al.* 1992). STSs, by the original definition, are developed from molecular fragments that contain repetitive DNA to produce a non-repetitive DNA fragment. STS-type markers are inherited co-dominantly, permitting easy transfer of markers between genetic maps of different crosses. This is in contrast to a dominant PCR-type marker that requires the generation of a new map for each cross (Thomas and Scott 1993). STS markers are independent of the specific method employed in the physical mapping that allows for the communication between laboratories. The need to store and distribute large number of clones was eliminated (Paran and Michelmore 1993). STS markers have been identified with barley (Tragoonrung *et al.* 1992), grapes (Thomas and Scott 1993), and wheat (Schachermayr *et al.* 1994; Talbot *et al.* 1994; Feuillet *et al.* 1995).

Alternately, sequenced characterized amplified regions (SCARs) are PCR-based markers that represent single, genetically defined loci that are identified with pairs of specific oligonucleotide primers and may contain high-copy, dispersed genome sequences within an amplified region (Paran and Michelmore 1993). SCARs share the advantages of STSs but are distinct in two aspects: 1) they can be used as both physical and genetic markers, and 2) within the amplified fragment they can contain repetitive DNA sequences. SCARs are primarily defined genetically and analyzed by PCR only. The uniqueness of SCARs is determined by the sequence and spacing of their primers, rather than by hybridization (Paran and Michelmore 1993). SCARs have been developed for several plant species including lettuce (Paran and Michelmore 1993; Kesseli *et al.* 1994; Witsenboer *et al.* 1995), common bean (Adam-Blondon *et al.* 1994) and tomato (Williamson *et al.* 1994).

A PCR technique that is time saving and generates many polymorphic bands on a polyacrylamide gel is Amplified Fragment Length Polymorphism (AFLP). The technique involves having the DNA restricted with one or two restriction enzymes; suitable adapters are ligated, and selectively amplified with different primer combinations (Zabeau and Vos 1993). The major advantage of AFLP is the capacity of showing many polymorphic bands in one lane. AFLP marker analysis is not as easy as RAPDs, but more efficient than RFLP (Becker *et al.* 1995). A comparison of RFLP, RAPD, and AFLP markers in soybean germplasm demonstrated that all three assays have different properties. The choice of assay to be used should be based on informativeness and ease of genotyping (Powell *et al.* 1996). Whatever assay is used to identify the fragment of choice it still will need to be cloned.

YAC and BAC Cloning

Conventional cloning techniques are incapable of cloning large fragments of genomic DNA into a vector. When insert sizes needed are at least 100-kb, the cloning choices are yeast artificial chromosome (YACs) (Burke *et al.* 1987) and bacterial artificial chromosome (BACs) (Shizuya *et al.* 1992). Cloning fragments greater than 1-Mb is possible with YACs.

YACs were developed with the ability to maintain large DNA fragments as linear chromosomes in yeast when YAC vectors function as plasmids in *E. coli.* YACs can be used in conjunction with molecular markers such as RFLPs, RAPDs, and AFLPs to aid in chromosome walking or landing. Several YAC libraries have been constructed for a large number of plant and animal species. The isolation of the cloned DNA from the yeast host is neither simple nor rapid. The YAC cloning system disadvantages are the abundance of chimeric clones, unstable clones, and

the tendency to delete internal regions from their inserts (Ling *et al.* 1993; Monaco and Larin 1994).

BACs are constructed by the F-factor derived vector system in *E. coli,* which allows the clones to be in the form of a plasmid in the host. This construction allows the BAC DNA to be purified by standard plasmid purification. BAC vectors are maintained as single copy supercoiled plasmid which gives lower levels of chimerism, increased stability over high copy vectors, and easier to generate libraries. Using media selection identifies positive clones. BACs are simpler to manipulate and to isolate insert DNA from than YACs (Shizuya *et al.* 1992). The disadvantage of BACs is the DNA yield from the clones is low due to the F- factor replicating only allowing one or two copies per cell (Monaco and Larin 1994).

New techniques are being developed and are used to continue to gain knowledge of the various genomes. Many of the molecular techniques described are used separately or in combination to study and dissect the genome of interest.

Bibliography

Adam-Blondon, A. F., M. Sevignac, H. Bannerot, and M. Dron. (1994). SCAR, RAPD, and RFLP markers linked to a dominant gene (*Are*) conferring resistance to anthracnose in common bean. Theor. Appl. Genet. 88:865-870.

Arumuganathan, K. and E. D. Earle. (1991). Nuclear DNA content of some important plant species. Plant-Mol-Biol-Rep-ISPMB. Athens, Ga.: International Society for Plant Molecular Biology, University of Georgia 9(3):208-218.

Becker,J., P. Vos, M. Kuiper, F. Salamini, and M. Heun. (1995). Combined mapping of AFLP and RFLP markers in barley. Mol. Gen. Genet. 249:65-73.

Bennett, M. D. and J. B. Smith. (1976). Nuclear DNA amounts in angiosperms. Philos. Trans. R. Soc. London Ser. B 274:227-274.

Binelli, G. and G. Bucci. (1994). A genetic linkage map of *picea abies* Karst., based on RAPD markers, as a tool in population genetics. Theor. Appl. Genet. 88:283-288.

Black IV, W. C., N. M. DuTeau, G. J. Puterka, J. R. Nechols, and J. M. Pettorini. (1992). Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera:*Aphididae*). Bulletin of Entomological Research 82:151-159.

Bothmer, R. von, N. Jacobsen, C. Baden, R. B. Jørgensen, and I. Linde-Laursen. (1991). Introduction In: An ecogeographical study of the genus *Hordeum*. Systematic and ecogeographical studies on crop gene pools 7. International Board for Plant Genetic Resources, Rome, pp. 1-15.

Bonnier, F. J. M., K. Reinink, and R. Groenworld. (1994). Genetic analysis of *Lactuca* accessions with major gene resistance to lettuce downy mildew. Phytopathology 84:462-468.

Briggs, D. E. (1978). The growth of the barley plant. In: Barley. Briggs DE, ed. 222-280. London: Chapman and Hall.

Burke, D. T., G. F. Carle, and M. V. Olson. (1987). Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806-812.

Chalmers, K. J., U. M. Barua, C. A. Hackett, W. T. B. Thomas, R. Waugh, and W. Powell. (1993). Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. Theor. Appl. Genet. 87:314-320.

Chaparro, J. X., D. J. Wemer, D. O'Malley, and R. R. Sederoff. (1994). Targeted mapping and linkage analysis of morphological isozyme and RAPD markers in peach. Theor. Appl. Genet. 87:805-815.

Chapco, W., N. W. Ashton, R. K. B. Martel, N. Antonishyn, and W. L. Crosby. (1992). A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. Genome 35:569-574.

Cocking E. C. (1990). Plant genetic manipulation for crop improvement: the role of cell and tissue culture. In: Symposium of Plant Biotechnology, EBC Sypmosium on Plant Biotechnology, Helsinki. pp. 50-58.

Delourme, R., A. Bouchereau, N. Hubert, M. Renard and B. S. Landry. (1994). Identification of RAPD markers linked to a fertility restorer gene for the *Ogura* radish cytoplasmic male sterility of Rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 88:741-748.

Demeke, T., R. P. Adams and R. Chibbar. (1992). Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. Theor. Appl. Genet. 84:990-994.

DeScenzo, R. A., R. P. Wise, and M. Mahadevappa. (1994). High resolution mapping of the *Hor1/Mla/Hor2* region on chromosome 5S in barley. Molecular Plant-Microbe Interactions 7(5):657-665.

Farrara, B. F., T. W. Ilott, and R. W. Michelmore. (1987). Genetic analysis of factors for resistance to downy mildew (*Bremia lactucae*) in species of lettuce (*Lactuca sativa* and *L. serriola*). Plant Pathol. 36:499-514.

Feuillet, C., M. Messmer, G. Schachermayr, and B. Keller. (1995). Genetic and physical characterization of the *LR1* leaf rust resistance locus in wheat (*Triticum aestivum* L.). Mol. Gen. Genet. 248:553-562.

Flor, H. H. (1956). Host-parasite interactions in flax rust-its genetics and other implications. Phytopathology 45:680-685.

Flor, H. H. (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.

Freialdenhoven, A.. C., B. Scherag, K. Hollricher, D. B. Collinge, H. Thordal-Christensen, and P. Schulze-Lefert. (1994). *Nar-1* and *Nar-2*, Two loci required for *Mla*12-specified race-specific resistance to powdery mildew in barley. Plant Cell 6:983-994.

Freialdenhoven, A., C. Peterhansel, J. Kurth, and F. Kreuzaler. (1996). Identification of genes required for the function of non-race-specific *mlo* resistance to powdery mildew in barley. The Plant Cell 8:5-14.

Giese, H., A. G. Holm-Jensen, H. P. Jensen, and J. Jensen. (1993). Localization of the *Laevigatum* powdery mildew resistance gene to barley chromosome 2 by the use of RFLP markers. Theor. Appl. Genet. 85(6/7):897-900.

Giovannoni, J. J., R. A. Wing, M. W. Ganal, and S. D. Tanksley. (1991). Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acid Research 19(23):6553-6558.

Görg, R., K. Hollricher, and P. Schulze-Lefert. (1993). Functional analysis and RFLP-mediated mapping of the *Mlg* resistance locus in barley. The Plant Journal 3(6):857-866.

Graner, A., A. Jahoor, J. Schondelmaier, H. Siedler, K. Pillen, G. Fischbeck, G. Wenzel, and R. G. Herrmann. (1991). Construction of an RFLP map of barley. Theor. Appl. Genet. 83:250-256.

Guthrie, P. A. I., C. W. Magill, R. A. Frederiksen, and G. N. Odvady. (1992). Random amplified polymorphic DNA markers: A system for identifying and differentiating isolates of *Colletotrichum graminicola*. Phytopathology 82(8):832-835.

Haley, S. D., P. N. Miklas, J. R. Stavely, and J. D. Kelly. (1993). Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor. Appl. Genet. 86:505-512.

Heun, M. and T. Helentjaris. (1993). Inheritance of RAPDs in F1 hybrids of corn. Theor. Appl. Genet. 85:961-968.

Heun, M., A. E. Kennedy, J. A. Anderson, N. L. V. Lapitan, M. E. Sorrells and S. D. Tanksley. (1991). Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437-447.

Hinze, K., R. D. Thompson, E. Ritter, F. Salamini and P. Schulze-Lefert. (1991). Restriction fragment length polymorphism-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*). Proc. Natl. Acad. Sci. 88:3691-3695.

Horn, P. and A. Rafalski. (1992). Nondestructive RAPD genetic diagnostic of microspore-derived *Brassica* embryos. Plant Molecular Biology Reporter 10(3):285-293.

Hulbert, S. H. and J. L. Bennetzen. (1991). Recombination at the *Rp1* locus of maize. Mol. Gen. Genet. 226(3):377-382.

Hulbert, S. H. and R. W. Michelmore. (1985). Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa*). Theor. Appl. Genet. 70:520-528.

Hunt, G. J. and R. E. P. Jr. (1992). Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honeybee. Theor. Appl. Genet. 85:15-20.

Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White, Eds. (1990). PCR Protocols A Guide to Methods and Applications. San Diego, CA, Academic Press, Inc.

Johnson, E., P. N. Miklas, J. R. Stavely, and J. C. Martinez-Cruzado. (1995). Coupling- and repulsion-phase RAPDs for markers-assisted selection of PI 181996 rust resistance in common bean. Theor. Appl. Genet. 90:659-664.

Jørgensen, J. H. (1994). Genetics of powdery mildew resistance in barley. Genome 13:492-498.

Jørgensen, J. H. and H. P. Jensen. (1976). New linkage data with the *ml-o* locus on barley chromosome 4. Barley Genet. Newsletter 6:39-41.

Jørgensen, J. H. and J. G. Moseman. (1972). Recombination at the *Mla* locus in barley conditioning resistance to *Erysiphe graminis* f. sp. *hordei.* Can. J. Genet. Cytol. 14:43-48.

Kesseli, R. V., I. Paran, and R. W. Michelmore. (1994). Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136:1435-1446.

Kintzios, S., A. Jahoor, and G. Fischbeck. (1995). Powdery-mildew-resistance genes *Mla29* and *Mla32* in *H. spontaneum* derived winter-barley lines. Plant Breeding 114:265-266.

Kleine, M., W. Michalek, A. Graner, R. G. Herrmann, and C. Jung. (1993). Construction of barley (*Hordeum vulgare* L.) YAC library and isolation of a *Hor1*-specific clone. Mol. Gen. Genet. 240:265-272.

Kleinhofs, A., A. Kilian, M. A. S. Maroof, R. M. Biyashev, P. Hayes, F. Q. Chen, N. Lapitan, A. Fenwick, T. K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Kudrna, J. Bollinger, S. J. Knapp, B. Liu, M. Sorrells, M. Heun, J. D. Franckowiak, and D. Hoffman. (1993). A molecular, isozyme, and, morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86:705-712.

Koller, B., A. Lehmann, J. M. McDermott, and C. Gessler. (1993). Identification of apple cultivars using RAPD markers. Theor. Appl. Genet. 85:901-904.

Lelner, A., M. A. Campbell, N. C. Wheeler, T. Poykko, J. Glossl, J. Kreike, and D. B. Neale. (1995). Identification of a RAPD marker linked to the pendula gene in Norway spruce (*Picea abies* (L.) Karst. f. *pendula*). Theor. Appl. Genet. 91:1092-1094.

Ling, L. L., N. S. F. Ma, D. R. Smith, D. D. Miller, and D. T. Moir. (1993). Reduced occurrence of chimeric YACs in recombination deficient hosts. Nucleic Acids Research 21:6045-6046.

Mahadevappa, M., R. A. DeScenzo, and R. P. Wise. (1994). Recombination of alleles conferring specific resistance to powdery mildew at the *Mla* locus in barley. Genome 37:460-468.

Maisonneuve, B., Y. Bellec, P. Anderson, and R. W. Michelmore. (1994). Rapid mapping of two genes for resistancea to downy mildew from *Lactuca serriola* to existing clusters of resistance genes. Theor. Appl. Genet. 89(1):96-104.

Michel, M. (1995). Übertragung von *H. bulbosum*- Resistenzgenen gegenüber Mehltau und Gerstengelbmosaikvirus in die Kulturgerste. Vortr Pflanzenzüchtg 31:78-79.

Michelmore, R. W., I. Paran, and R. V. Kesseli. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA. 88:9828-9832

Miklas, P. N., J. R. Stavely, and J. D. Kelly. (1993). Identification and potential use of a molecular marker for rust resistance in common bean. Theor. Appl. Genet. 85:745-749.

Mohan, M., S. Nair, J. S. Bentur, U. P. Rao, and J. Bennett. (1994). RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*). Theor. Appl. Genet. 87:782-788.

Monaco A. P., and Z. Larin. (1994). YACs, BACs, PACs, and MACs: artificial chromosomes as research tools. Trends in Biotechnology 12:280-286.

Mouzeyar, S., P. Roeckel-Drevet, L. Gentzbittel, J. Philippon, D. T. D. Labrouhe, F. Vear, and P. Nicolus. (1995). RFLP and RAPD mapping of the sunflower *Pl1* locus for resistance to *Plasmopara halstedii* race 1. Theor. Appl. Genet. 91:733-737.

Oh, B. J., R. A. Frederiksen, and C. W. Magill. (1994). Identification of molecular markers linked to head smut resistance gene (*Shs*) in sorghum by RFLP and RAPD. Phytopathology 84(8):830-833.

Paran, I. and R. W. Michelmore. (1993). Development of reliable PCR-based markers linked to downy mildew resistance gene in lettuce. Theor. Appl. Genet. 85:985-993.

Paran, I., R. V. Kessli, and R. W. Michelmore. (1991). Identification of RFLP and RAPD markers to downy mildew resistance genes in lettuce with near-isogenic lines. Genome 34:1021-1027.

Penner, G. A., A. Bush, R. Wise, W. Kim, L. Domier, K. Kasha, A. Laroche, G. Scales, S. J. Molnaer, and G. Fedak. (1993). Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR methods and applications, Cold Spring Harbor Laboratories. 2:341-345.

Pickering, R. A., A. M. Hill, M. Michel, and G. M. Timmerman-Vaughan. (1995). The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L. to barley (*H. vulgare* L.) chromosome 2 (2I). Theor. Appl. Genet. 91:1288-1292.

Pickering, R. A., W. F. Rennie, and M. G. Cromey. (1987). Disease resistance material available from the wide hybridization program at DSIR. Barley Newsletter 31:248-250.

Poulsen, D. M. E., R. J. Henry, R. P. Johnston, J. A. G. Irwin, and R. G. Rees. (1995). The use of bulked segregant analysis to identify a RAPD marker linked to leaf rust resistance. Theor. Appl. Genet. 91:270-273.

Powell, W., M. Morgante, C. Andre, M. Hanafrey, J. Vogel, S. Tingey, and A. Rafalski. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2:225-238.

Pryor, T. (1987). The origin and structure of fungal disease resistance genes in plants. Trends Genet. 3(6):157-161.

Rayapati, P.J., J. W. Gregory, M. Lee, and R. P. Wise. (1994). A linkage map of diploid *Avena* based on RFLP loci and a locus conferring resistance to nine isolates of *Puccinia coronata* var. *avenae*. Theor. Appl. Genet. 89:831-837.

Reiter, R. S., J. G. K. William, K. A. Feldmann, J. A. Rafalski, S. V. Tingey, and P. A. Scolnik. (1992). Global and local genome mapping in *Arabidopis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acad. Sci. USA. 89(4):1477-1481.

Santos, J. B. d., J. Nienhuis, P. Skroch, J. Tivang, and M. K. Slocum. (1994). Comparison of RAPD and RFLP genetic markers in determing genetic similarity among *Brassica oleracea* L. genotypes. Theor. Appl. Genet. 87:909-915.

Saxena, K. M. S and A. L. S. Hooker. (1968). On the structure of a gene for disease resistance in maize. Proc. Natl. Acad. Sci. U.S.A. 61:1300-1305.

Schachermayr, G., H. Siedler, M. D. Gale, H. Winzeler, M. Winzeler, and B. Keller. (1994). Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theor. Appl. Genet. 88:110-115.

Shin, J. S., S. Chao, L. Corpuz, and T. Blake. (1990). A partial map of the barley genome incorporating restriction fragment length polymorphism, polymerase chain reaction, isozyme, and morphological marker loci. Genome 33:803-810.

Shizuya, H., B. Birren, U. J. Kim, V. Mancino, T. Slepak, Y. Tachiiri, and M. Simon. (1992). Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc. Natl. Acad. Sci. USA. 98:8794-8797.

Skroch, P. and J. Nienhuis. (1995). Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appl. Genet. 91:1086-1091.

Søgaard, B. and P. von Wettstein-Knowles. (1987). Barley: genes and chromosomes. Carlsberg Res. Commun. 52:123-196.

Sudupak,M. A., J. L. Bennetzen, and S. H. Hulbert. (1993). Unequal exchange and meiotic instability of disease-resistance genes in the *Rp1* region of maize. Genetics 133(1):119-125..

Talbot, L. E., N. K. Blake, P. W. Chee, T. K. Blake, and G. M. Magyar. (1994). Evaluation of "sequence-tagged site" PCR products as molecular markers in wheat. Theor. Appl. Genet. 87:789-794.

Thomas, M. R. and N. S. Scott. (1993). Microsatellites repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86:985-990.

Tinker, N. A., M. G. Fortin, and D. E. Mather. (1993). Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor. Appl. Genet. 85:976-984.

Tragoonrung, S., V. Kanazin, P. M. Hayes, and T. K. Blake. (1992). Sequence-tagged-site-facilitated PCR for barley genome mapping. Theor. Appl. Genet. 84:1002-1008.

Vierling, R. A., Z. Xiang, C. P. Joshi, M. L. Gilbert, and H. T. Nguyen. (1994). Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. Theor. Appl. Genet. 87:816-820.

Wang, G.-L. and A. H. Paterson. (1994). Assessment of DNA pooling strategies for mapping of QTLs. Theor. Appl. Genet. 88:355-361.

Wechter, W. P., M. P. Whitehead, C. E. Thomas, and R. A. Dean. (1995). Identification of a randomly amplified polymorphic DNA marker linked to the *Fom 2 Fusarium* wilt resistance gene in muskmelon *MR-1.* Phytopathology 85(10):1245-1249.

Welsh, J., K. Chada, S. S. Dalal, R. Cheng, D. Ralph, and M. McClelland. (1992). Arbitrarily primed PCR fingerprinting of RNA. Nucleic Acid Research 20(19):4965-4970.

Wiberg, A. (1974). Genetical studies of spontaneous sources of resistance to powdery mildew. Hereditas 77:89-148.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingley. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18(22):6531-6535.

Williamson, V. M., J.-Y. Ho, F. F. Wu, N. Miller, and I. Kaloshian. (1994). A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor. Appl. Genet. 87:757-763.

Wise, R. P. and A. H. Ellingboe. (1983). Infection kinetics of *Erysiphe graminis* f. sp. *hordei* on barley with different alleles at the *Ml-a* locus. Phytopathology 73:1220-1222.

Wise, R. P. and A. H. Ellingboe. (1985). Fine structure and instability of the *Ml-A* locus in barley. Genetics 111:113-130.

Wise, R. P., M. Lee, and P. J. Rayapati. (1996). Recombination within 5 cM region in diploid *Avena* reveals multiple specificities conferring resistance to *Puccinia coronata*. Phytopathology 86:340-346.

Witsenboer, H., R. V. Kesseli, M. G. Fortin, M. Stanghellini, and R. W. Michelmore. (1995). Sources and genetic structure of a cluster of genes for resistance to three pathogens in lettuce. Theor. Appl. Genet. 91:178-188.

Xu, J. and K. J. Kasha. (1992). Transfer of a dominant gene for powdery mildew resistance and DNA from *Hordeum bulbosum* into cultivated barley (*H. vulgare*). Theor. Appl. Genet. 84:771-777.

Xu, J. and J. W. Snape. (1989). The resistance of *Hordeum bulbosum* an its hybrids with *H. vulgare* to common fungal pathogens. Euphytica 41:273-276.

Yu, L. -X. and H. T. Nguyen. (1994). Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theor. Appl. Genet. 87:668-672.

Zabeau, M. and P. Vos. (1993). Selective restriction fragment amplification: a general method for DNA fingerprinting. Publication number:0 543 858 A1.

RAPD-DERIVED MARKERS LINKED TO THE *Mla* RESISTANCE GENE CLUSTER IN BARLEY

A paper to be submitted to the Journal of Agricultural Genomics

Karin Sue Gobelman-Werner and Roger Wise

INTRODUCTION

As one of the important small grains grown in the world, barley (*Hordeum vulgare* L.) is also one the oldest cultivated crops. Barley is primarily grown in northwestern Europe, a few selected areas in North and South America, Australia and Asia. When grown in a cool, wet climate, barley can be highly susceptible to powdery mildew, which is caused by the obligate fungal pathogen, *Erysiphe graminis* DC. Merat f. sp. *hordei* Em. Marchal. An effective control measure is to acquire resistance to the fungal agent.

Many genes, designated *Ml*, have been identified which confer resistance to powdery mildew in barley. At least 30 *Mla* specificities in barley have been identified which demonstrate resistance to *E. graminis* (Jørgensen and Moseman 1972; Jørgensen and Jensen 1976; Hinze *et al.* 1991; Görg *et al.* 1993; Jørgensen 1994; Kintzios *et al.* 1995). RFLP markers have been described that are linked to particular resistance specifities, but these markers are not close enough to clone the resistance specificity. Additional markers were necessary to saturate the region surrounding the *Mla* resistance-gene cluster efficiently clone the resistance gene.

In this report, we describe the identification of additional markers linked to the *Mla* locus. These markers help saturate the chromosomal region near *Mla* and produce a more detailed map. These markers were also used to screen large insert barley YAC library (Kleine *et al.* 1993; 1997), from the cultivars Ingrid and Franka.

Materials and Methods

Barley Germplasm

The high resolution mapping population has been described previously (Mahadevappa *et al.* 1994; DeScenzo *et al.* 1994). The Franger- (C.I. 16151) and Rupee- (C.I. 16155) derived isogenic lines (Wise and Ellingboe 1983) contains the *Mla6* + *Mla14* and the *Mla13* + *Ml-Ru3* resistance specificities, respectively. A total of 286 barley lines were identified to be recombinant between and homozygous at the *Hor1* and *Hor2* loci from 3,600 F_1 gametes (1800 F_2 seed).

A 3-cM window that encompasses the *Mla* gene cluster was defined by the recombination breakpoints in our high-resolution barley population (DeScenzo *et al.* 1994). Three subsets of this population were established from the high-resolution barley population to help focus on the region of interest. The subsets were pools for bulked segregant analysis, a recombinant interval mapping population, and a focused high-resolution population. Bulked segregant analysis was performed on pools of DNA from 14-15 homozygous recombinant lines. These pools were homogeneous for either the *Mla6* + *Mla14* (pool A) or the *Mla13* + *Ml-Ru3* (pool B) resistance specificities. The recombinant mapping population and focused population were used to determine the RAPD marker location in the barley genome. The recombinant interval mapping population consists of two reciprocal recombination events per mapping interval between *Hor1* and *Hor2*. The focused high-resolution population consisted of 89 individuals that contain recombination breakpoints in the *Xmwg036- Xmwg068* interval.

Powdery Mildew Resistance Screening

The three powdery mildew isolates 5874, A27, and R63 were used to inoculate barley accessions to screen for infection type. Three seeds per accession were planted in a 20 X 30 cm flat and grown in greenhouse conditions until inoculated. Seedlings were inoculated 8-10 days after emergence when they were approximately 3 inches high. The inoculated seedlings were placed in a growth chamber (Percivial, Boone, IA) and sustained an 18 hour photoperiod at 18 °C. Disease development was evaluated at 7 days after inoculation (Wise and Ellingboe 1983; Mahadevappa *et al.* 1994). The infection types 0, 1, or 2 were considered resistant reactions while the infection types 3 or 4 were considered susceptible reactions (Wise and Ellingboe 1983).

Plant DNA Isolation

Barley DNA was extracted from 0.5 g samples of frozen tissue using a modified hexadecyl trimethylammonium bromide (CTAB) method for fresh tissue (Bush *et al.* 1994; Wise and Schnable 1994) and quantified using GeneQuant II (Pharmacia, Piscataway, NJ). DNA was diluted in sterile double distilled water to a final concentration of 50 ng/µl.

RAPD and STS Analysis

A total of forty Operon (Operon Technologies Inc., Alameda, CA) and 699 University of British Columbia (Carlson; Oligonucleotide Synthesis Laboratory, University British Columbia, Vancouver, Canada) arbitrary nucleotide sequences were used in this analysis.

The PCR amplification was performed in a volume of 25 µl with a 1x reaction buffer supplied by the manufacturer [20 mM Tris-HCl (pH 8.4), 50 mM KCl], 1.5 mM $MgCl_2$, 0.001% gelatin, 0.1 mM each of dNTP, 50 ng of genomic DNA, 0.625 units of Taq DNA polymerase (Gibco BRL Life Technologies, Rockville, MD) and either 5 µM decamer RAPD- or 20 µM STS-primers. The PCR amplifications were performed in a PTC-100 programmable thermocycler (MJ Research Inc., Watertown, MA). Amplification programs were as follows: RAPD: one cycle for 1 minute at 94 °C; 44 cycles for 5 seconds at 94 °C, 30 seconds at 36 °C, 1 minute at 72 °C; with a final extension of 9 minutes at 72 °C, STS: one cycle for 3 minute at 94 °C, 29 cycles for 30 seconds at 94 °C, 1 minute at 60 °C, 1 minute at 72 °C; with a final extension of 4 minutes at 72 °C. The amplification products were resolved by electrophoresis at 80 volts for 4 hours on a 2% thin (3 mm) agarose gel containing 1 X TBE buffer (0.089 M Tris, 0.089 M Borate, 0.002 M EDTA) (Sambrook *et al.* 1989).

Cloning of RAPD Fragment

A modified method of re-amplify the band of interest was used (Hengen 1995). A single plug of agarose was removed from the RAPD primer product detecting a polymorphism with the small end of a pasteur pipette and placed in 100 µl sterile double distilled water (ddH_2O) to elute at 4 °C overnight. One µl of eluted DNA / ddH2O solution was used as template for re-amplification with the original 10-bp-long oligonucleotide primer. DNA inserts were purified using the modified NA45 membrane (Schleicher & Schuell, Keene, NH) extraction, blunt-end ligated into the *Eco*RV site of the pGem-T plasmid (Promega, Madison, WI), and transformed into the *E. coli* TB-1 host strain. The cloned inserts were either released by a double digest of *Sac*II and *Pst*I or by direct PCR amplification using

T7 (5'-AATACGACTCACTATAG-3') and M13 reverse (5'-GGAAACAGCTATGACCATG -3') primer pairs.

Sequence Analysis and STS Design

Sequencing and oligonucleotide synthesis were performed at the Iowa State University DNA Sequencing and Synthesis Facility. The nucleotide sequence of the cloned RAPD fragment was determined by a Perkin-Elmer Applied system ABI model 377 automated DNA sequencing system. The sequenced-tagged site (STS) oligonucleotides were synthesized on a Perkin Elmer Applied System ABI model394 DNA/RNA synthesizer. Sequence data was entered into the program AutoAssembler (Perkin Elmer, Modesto, CA). Sequence data was assembled using the "Assemble" command (10-bp pair overlap and 5% error).

Linkage Analysis

RAPD marker data was entered into a Map Manager V2.5 (copyright 1988-1994, K. Manly, Roswell Park Cancer Institute) file containing previous marker data for the *Hor1-Hor2* high resolution mapping population (DeScenzo *et al.* 1994). MAPMAKER readable files were created using the Export for MAPMAKER option. MAPMAKER Macintosh V2.0 (copyright 1993 E.I. DuPont de Nemours and Co., Lander *et al.* 1987) was used in the analysis the segregation data. Markers were grouped together using the "Group" command (minimum LOD of 8.0) and ordered relative to the resistance locus using the "Try" command. Recombination frequencies were converted to genetic distances using Kosambi mapping function.

RESULTS

RAPD Analysis

New markers tightly linked to the *Mla* gene cluster must have the following criteria. The markers must be discrete and polymorphic between the barley accessions of C.I. 16151 (*Mla6* + *Mla14*) and C.I. 16155 (*Mla13* + *Ml-Ru3*), the parents of our high-resolution mapping population. The markers must map within 0.2 cM of the *Mla* gene cluster to be useful for map-based gene isolation. Allele-specific primers developed from these markers should amplify the identical fragment from Ingrid (Büschges *et al.* 1997) and Franka (Kleine *et al.* 1993; 1997), the cultivars used to construct barley YAC libraries, but not the AB1380 yeast host strain. To screen the Morex BAC library via high-density filter hybridization (Yu *et al.* 2000), the amplified product should hybridize to a low-copy fragment in the barley genome.

Seven hundred thirty-nine arbitrary RAPD primers were evaluated to identify DNA polymorphisms between the C.I. 16151 (*Mla6* + *Mla14*) and C.I. 16155 (*Mla13* + *Ml-Ru3*) and pools. Zero to eighteen DNA fragments were amplified for each primer for a total of 4819 discrete amplified products (2410 from C.I. 16151 and 2409 from C.I. 16155) (Appendix A). Three primers amplified discrete polymorphic DNA fragments that were positioned between *Hor1* and *Hor2* on chromosome 5 (1H).

Primer OPA-10 (GTGATCGCAG) produced a 1500-bp, C.I. 16155-specific fragment, designated $OPA\text{-}10_{1500}$, was positioned between *Hor1* and *XChs3*. Primer UBC465 (GGTCAGGGCT) amplified a 950-bp, C.I. 16155-specific fragment, designated $UBC465_{950}$, that mapped between *XChs3* and *Xmwg068*. Primer UBC165 (GAAGGCACTG) amplified a 1626-bp, C.I. 16155-specific fragment, designated $UBC165_{1626}$, that mapped within the *Xmwg036-Xmwg068* interval. The

UBC165$_{1626}$ –derived fragment was fine mapped using the 89 recombinants in the *Xmwg036-Xmwg068* interval. Of the 50 recombinants between X*bcd249.1* and *Mla6,* twelve had crossovers between *Mla6* -*UBC165*$_{1626}$, which equaled a distance of 0.28 cM proximal to *Mla6.*

Cloning of RAPD Fragments

The 1626-bp *UBC165*$_{1626}$-derived fragments was cloned and used as hybridization probe to test for copy number. Unfortunately, the cloned *UBC165*$_{1626}$ fragment hybridized to a highly repetitive fraction of the barley genome and therefore was not be used as a RFLP marker. The 1626-bp of the *UBC165*$_{1626}$-derived fragment was digested with the restriction endonucleases *Pst*I, *Sac*II, *Alu*I, *Hinc*II, *Hha*I *Hae*III, *Hin*fI and *Sau*3A. The digested fragments were size fractionated via agarose gel electrophoresis, transferred to nylon, and hybridized with total genomic barley to identify the repetitive regions. The *UBC165*$_{1626}$-derived fragment released four non-repetitive fragments with the *Hinc*II restriction endonuclease. These sub-fragments were purified and used as individual hybridization probes onto small strip blots of *Hin*dIII digested barley DNA. Unfortunately, all of these *UBC165*$_{1626}$-derived sub-fragments also hybridized to a middle-repetitive fraction, and therefore, were too repetitive for further RFLP analysis.

Generation of STS Markers

Twenty specific PCR primers were developed from the *UBC165*$_{1626}$ sequence (Table 1). The placement of the individual primers identified from *UBC165*$_{1626}$-derived fragment is detailed in Figure 1. The original UBC165 RAPD

Table 1. Oligonucleotide primers derived from the *UBC165*$_{1626}$ sequence

Primer abbreviation	Sequence 5' to 3'	Sequence length	ISU DSSF designation[b]
P0[a]	GAAGGCACTGAATCGTTGATGG	22	KW0511
P53	CTTTGTATTGCTTTGCTATCTG	22	KW0508
P174	TCGGTTTGGCTTTTTTCCTAATCC	24	KW0513
P174RC	GGATTAGGAAAAAAGCCAACCGA	24	KW0084
P203	GTACAAGGGAAACGTGATTA	20	KW1349
P203RC	TAATCACGTTTCCCTTGTAC	20	KW0509
P260RC	GGGAATGAGCCGAGCCGAAC	20	KW0507
P300	ACGAGGCGGGCGGTGGAGGAGGAG	24	KW1816
P300RC	CTCCTCCTCCACCGCCCGCCTCGT	24	KW0510
P451	CAATGGTGGTGTGGACTCTAGG	22	KW1817
P544	ACCATATACTAGAGGCAACACAAA	24	KW0900
P544RC	TTTGTGTTGCCTCTAGTATATGGT	24	KW0901
P671	TGAACAGTGGACTAGGCCTTGAAC	24	KW0902
P671RC	GTTCAAGGCCTAGTCCACTGTTCA	24	KW0903
P954	CAGTTTAGGGAAGTATTGCATC	22	KW0097
P954RC	GATGCAATACTTCCCTAAACTG	22	KW0512
P1034	GTCTTCCACATCTAATCACGGG	22	KW1362
P1034RC	CCCGTGATTAGATGTGGAAGAC	22	KW0086
P1373RC	GATTGGCTTCGCCGGCTCTTGAG	23	KW1361
P1585RC	TGGAATTCACTATACTGGACAC	22	KW1814

[a] Primers shown in bold amplify the *Fr1062* fragment.

[b] Designation given by the Iowa State University DNA Sequencing and Synthesis Facility (DSSF).

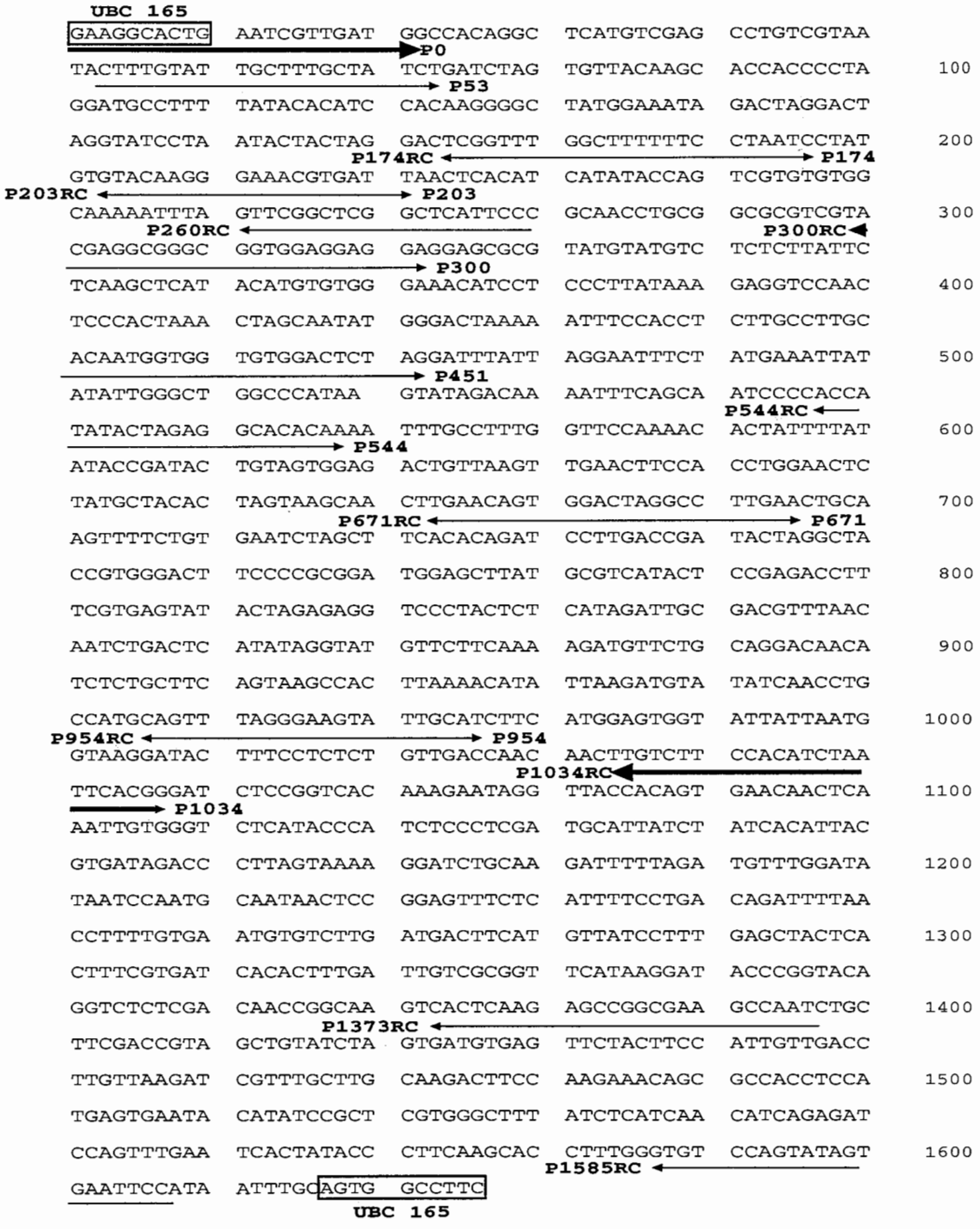

Fig. 1. *UBC165*$_{1626}$ sequence illustrating the position of 20 STS primers. Forward Boxes indicate the original location of the UMC165 RAPD primer. The bold arrows designate the P0 and P1034RC primers, which amplify the *Fr1062* fragment.

primer is identified at both the 5' and 3' ends. Various primers were used in combination to test for polymorphism between the C.I. 16151 (*Mla6* + *Mla14*) and C.I. 16155 (*Mla13* + *Ml-Ru3*) mapping parents. Figure 2 illustrates the different sizes of the fragments that were amplified throughout the $UBC165_{1626}$ sequence. The dotted lines indicate that the amplified product was C.I. 16151 specific, whereas, the solid lines designate that the product was amplified in both the C.I. 16151 and C.I. 16155. Primers that originate from opposite sides of the 174-nucleotide position (in bold) amplify polymorphic products, whereas, primers positioned on the same side result in monomorphic amplifications

The polymorphic primer combinations were used to position the $UBC165_{1626}$–derived STS markers relative to the *Mla* locus. Out of the 20 STS primer combinations tested, 13 primer pairs amplified fragments that cosegregated with the *Mla* using a low-resolution, interval mapping population. The same mapping pattern is evident although each STS produced a different size fragment as demonstrated in Figure 3 illustrates the interval mapping of the amplified products derived from two such STS primer pairs, *FR1062* and *FR1492.*

The 13 primer pairs that produced cosegregating fragments were tested on the eighty-nine recombinant lines in the *Xbcd249.1-Xmwg036* interval. Eight of these polymorphic fragments were accurately positioned. As shown in Figure 4, the primers that amplified *FR1062*, *FR106*, *Fr125*, *Fr226*, *Fr250*, *Fr860*, *Fr1350*, and *FR1492* were all positioned 0.28 cM distal to *Mla*, in contrast to the original proximal position of $UBC165_{1626}$ RAPD marker.

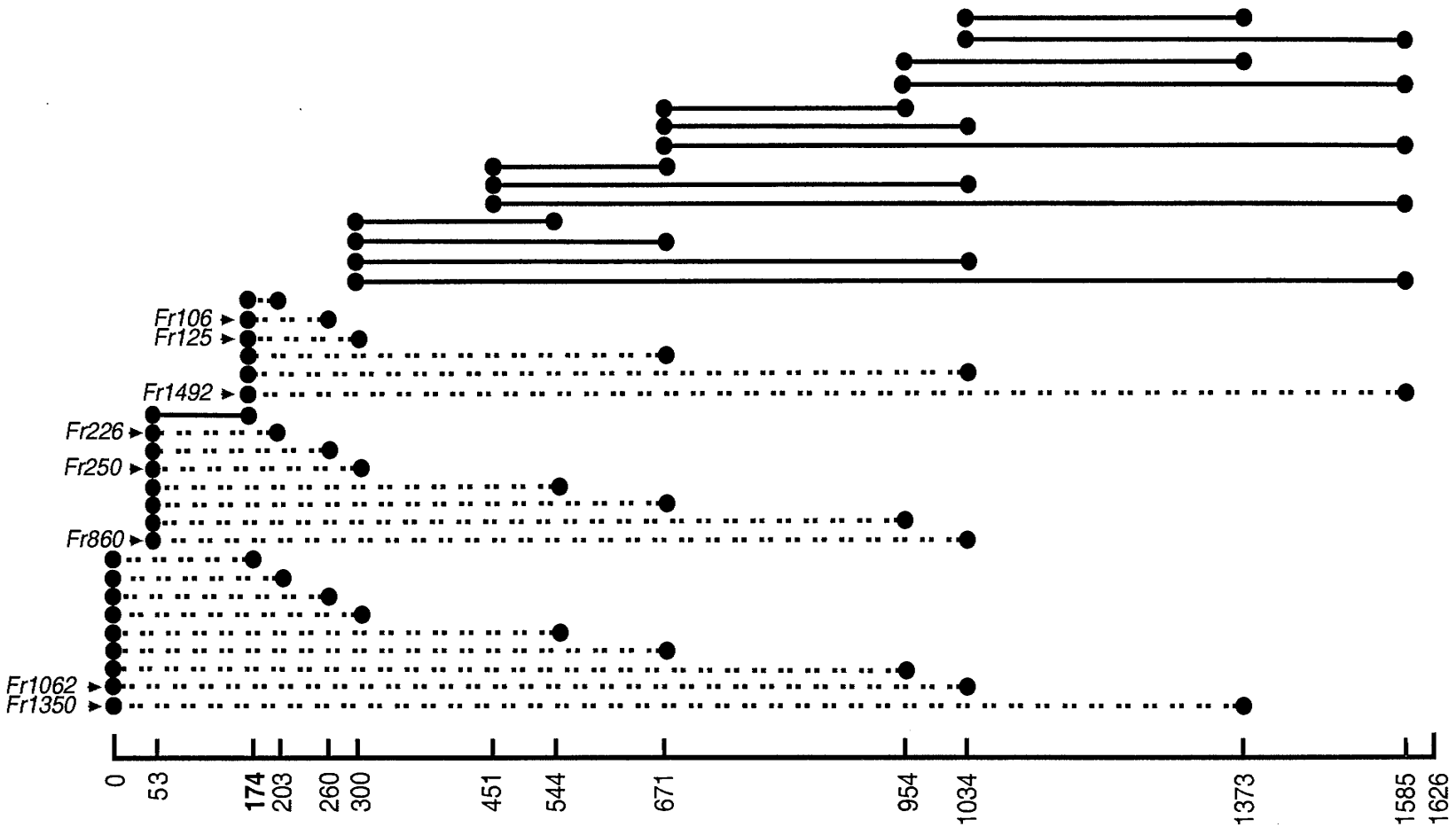

Fig. 2. Schematic of the products amplified with the *UMC165*$_{1626}$-derived STS primers. Primer locations are represented by filled circles. Polymorphic products are represented by dotted lines. Monomorphic products are represented by solid lines. STS markers are identified by grey arrowheads. Primers that originate from opposite sides of the 174-nucleotide position amplify polymorphic products. Primers positioned on the same side result in monomorphic amplifications.

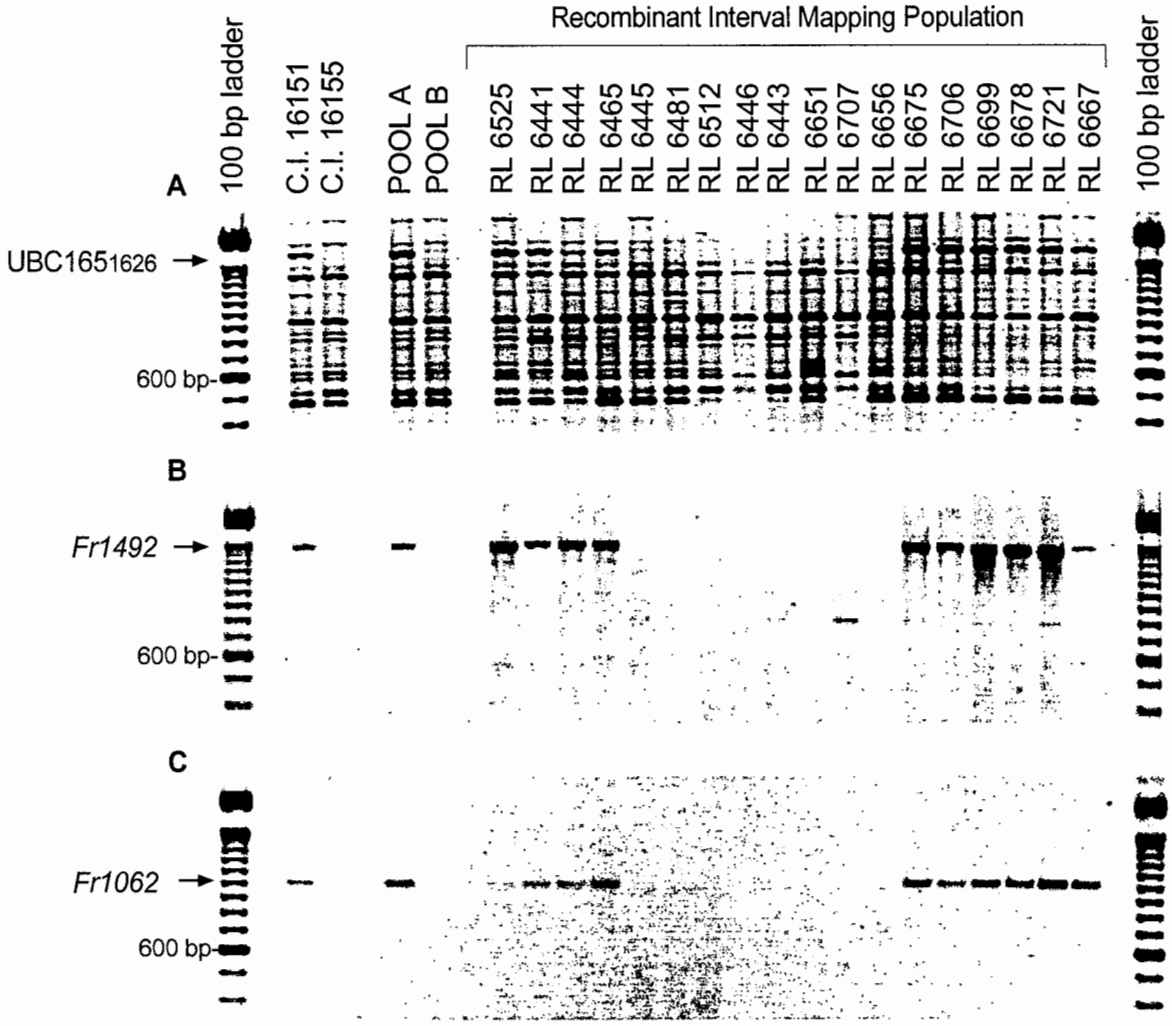

Fig. 3. Interval mapping of *UMC1651626, Fr1452,* and *Fr1062*. Pools A and B each contain genomic DNAs from 14 recombinant lines from our high-resolution mapping population. C.I. 16151 and Pool A contain the *Mla6* and *Mla14* resistance specificities, whereas, C.I. 16155 and Pool B contain the *Mla13* and *Ml-Ru3* resistance specificities.

A. The UMC165 RAPD primer amplified a 1626-bp product, designated *UMC1651626.* The arrow indicates the presence of the fragment in C.I. 16151 and all *Mla6*-containing recombinant lines.

B. The STS primers P174 + P1626 amplified a 1452-bp product, designated *Fr1452.* Products are present in C.I. 16151 and all *Mla6*-containing recombinant lines.

C. The STS primers P0 + P1034RC amplified a 1062-bp product, designated *Fr1062.* Products are present in C.I. 16151 and all *Mla6*-containing recombinant lines.

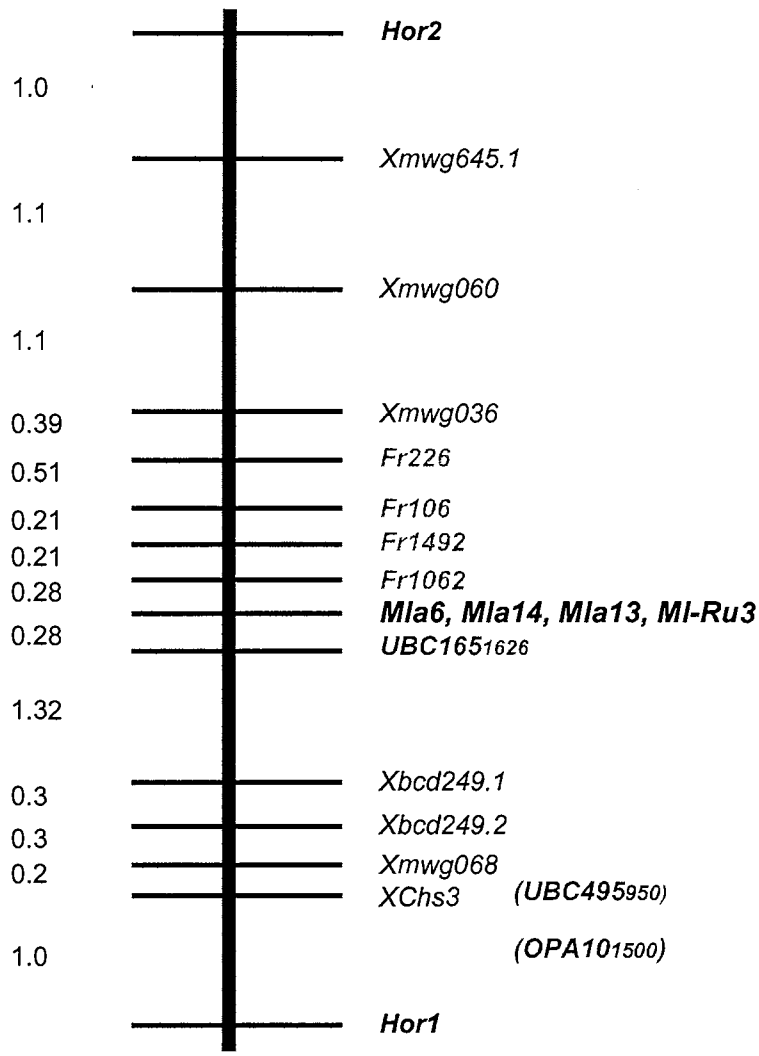

Fig. 4. Integration of the STS and RAPD markers into the high-resolution genetic map of the *Hor1* - *Hor2* region of barley chromosome 5 (1H). *Mla* specificities are in bold. The "*UBC*" prefixes designate RAPD primers from University of British Columbia and "*OPA*" prefixes designate RAPD primers from OperonTechnologies in bold grey. An "*Fr*" prefix designates new STS markers derived from the *UMC1651626* sequence (shown in light gray). An *X* prefix designates an RFLP marker; *mwg* markers are from Munich-Weihenstephan-Grunbach, *bcd* markers are from Cornell University, and *ciw* markers are from the Carnegie Institute of Washington.

The amplification product produced by the *FR1062* primer pair P0 + P1034RC consistently yielded the most stable map position. Therefore, P0 + P1034RC were tested on various accessions to see if the *FR1062* fragment could be amplified from Ingrid, Franka, and Morex, the cultivars used in the YAC and BAC library construction. The Ingrid and Franka cultivars, which are used in the construction of the Maltagen (YAC) library (Kleine *et al.* 1993; 1997), had the identical 1062-bp fragment as C.I. 16151. Unfortunately, the Morex cultivar, which was used to develop BAC library [Clemson University Genomic Institute (CUGI)], was not amplified. This information was used towards the molecular identification of the *Mla* cluster (Wei *et al.* 1999).

Inoculation with *E. graminis* f. sp. *hordei*

To investigation whether or not there was a correlation between infection type and the new STS markers, twenty-six different barley accessions were inoculated individually by three *E. graminis* isolates 5874, A27 and R63. The DNA from these accessions was used as PCR templates for four STS primer pairs. A comparison between the infection type and the PCR fragments amplified is illustrated in Table 2. All the STS primers amplify the same size fragment in all the accessions. The absence or slight amplification of a fragment seems to correlate with the infection type (IT) of susceptibility. A strong amplification of the *FR1062* fragment relates to the possibility of the presence of the *Mla6* resistance specificity. Preliminary testing of the STSs and powdery mildew isolates gives a tentative correlation between infection type (IT) and STS fragments. The absence or slight presence of a fragment seems to correlate the inadequacy of the barley accessions to have resistance to the 5874 isolate, which identifies the *Mla6* resistance

Table 2. Correlation between product produced from STSs and the infection type induced from three different powdery mildew isolates.

C.I. number	Accession	*Ml* specificity	STS primer pairs				Powdery mildew isolate		
			FR106 2	*FR1626*	*FR106*	*FR225*	5874	R63	R189
2330	Manchuria		-	-	-	-	4	4	4
16137	Algerian (R)	*Mla1*	+++	++	+	+++	1n	0	0-1
16138	Algerian (S)	*mla1*	+	-	+	-	4	4	4
16151	Franger (R)	*Mla6, Mla14*	+++	+	+	+	0	2-3n	2-3n
16155	Rupee (R)	*Mla13, Ml-Ru3,*	-	-	-	-	4	3	1
16149	Durani (R)	*Mla10* (Risø)	+++	-	+	+	4	ND	3-4
16150	Durani (S)	*mla10*	-	-	-	-	4	ND	4
16153	Long Glumes (R)	*Mla15, Mla7*	++	+	-	+++	4	4	1n
	Pallas	*Mla8*	+++	++	+	-	4	4	3-4n
	P03	*Mla6, Mla14*	+++	+	-	+	0	1-2n	1-2n
	P11	*Mla13, Ml-Ru3*	+	++	-	-	4	2-3n	1
16143	Kwan	*Mlk*	+	-	-	-	4	1-2n	2-3n
15229	Steptoe		-	-	-	+	4	3n	3-4
15773	Morex		-	-	+	-	4	4	3-4
	Harrington		++	+++	+	+	4	1	0-1
	Tr306		+	+++	+	+	4	4	3-4
P.I. 574293	Franka	*Mla6*	+++	+	+	++	0	1	1-2n
P.I. 406263	Igri	*Mla8*	+	-	-	-	4	2	3n
10063	Golden Promise	*Mla8*	-	+	-	++	4	2-3	3-4
14846	Sultan		-	-	-	-	1	ND	0
	Mc22		+++	+	+	+	2	ND	2-3
YAC library substrate	Ingrid		+	ND	+	+++	4	ND	ND

\+ denotes the presence of the band in C.I. 16151 parent
\- denotes the absence of the band in C.I. 16155 parent
ND denotes information was not determinate

specificity. R63 and A27 isolates do not identify this particular resistance specificity. There is no conclusive data to identify the different resistance specificities based on STSs. The pattern identified could be just a random occurrence. More studies are required to test this correlation.

DISCUSSION

The goal of this study was to identify additional markers in the *Mla* powdery-mildew resistance gene cluster using RAPD primers and bulked segregant analysis. Random amplified polymorphic DNA (RAPD) primers (Williams *et al.* 1990) used in conjunction with bulked segregant analysis (BSA) (Giovannoni *et al.* 1991) was used to saturate the chromosomal region of interest with new markers. RAPDs are arbitrary single ten base pair oligonucleotides, which detect polymorphic DNA fragments by polymerase chain reaction (PCR). These assays are fast, easy, requires small amount of DNA and do not require the use of radioactive isotopes. RAPDs have been used develop highly defined genetic maps and new molecular markers linked to the *Mi* nematode resistance gene of tomato (Klein-Lankhorst *et al.* 1991; Williamson *et al.* 1994), the *Lr9* leaf rust resistance gene of wheat (Schachermayr *et al.* 1994), and the *Pg3* oat stem rust gene (Penner *et al.* 1993). Bulked segregant analysis is a rapid and efficient method for the isolation of molecular markers linked to a defined genomic interval (Giovannoni *et al.* 1991; Michelmore *et al.* 1991). Bulked segregant analysis consists of pooling of homozygous individuals or individuals homogeneous at a single genetic point for the region of interest.

Of three RAPD primers that identified polymorphic fragments in the barley genome, only one, $UBC165_{1626}$, was 0.28 cM proximal to the resistance genes. The other two, $OPA\text{-}10_{1500}$ and $UBC465_{950}$, identified fragments mapping a greater genetic distance away from the *Mla* gene cluster. The $UBC165_{1626}$ RAPD fragment

is a dominant marker and contains highly repetitive DNA sequence. Only non-repetitive markers are useful in RFLP analysis, therefore, *UBC165*$_{1626}$ RAPD fragment was subsequently sequenced and individual primer pairs were established as STS markers.

Specific primers were designed across the*UBC165*$_{1626}$-derived fragment. The eight STS markers were discrete and polymorphic between the barley lines of C.I. 16151 and C.I. 16155. These eight markers were also positioned 0.28 cM distal to *Mla* gene cluster. *FR1062*, the most stable map position of the STS markers, PCR primers amplified the same fragment from Franka and Ingrid, the cultivars used in the construction of the Maltagen YAC library (Kleine *et al.* 1993; 1997).

The co-relationship between the STS fragments and the resistance specificities identified may be useful in identifying the presence of resistance specificities without screening the particular barley line. This would allow for a quick survey of the particular accession in question. Further testing is necessary to obtain an understanding of the relationship between an amplification of a fragment and the resistance specificities. Once the resistance specificities are cloned and STS primers are developed, the insertion of resistance specificities into the barley accessions will further the breeding practices.

CONCLUSION

RAPD analysis is a quick, efficient and non-radioactive method to identify new markers. Bulked segregant analysis used in conjunction with RAPD primers can be used to fill in gaps. We have used these techniques to identify additional closely linked markers to the *Mla* resistance gene cluster. The *UBC165*$_{1626}$ RAPD fragment was ineffective as a hybridization probe, but the STS primer pairs that

were developed were effective for PCR amplification, genetic mapping and YAC library screening. The integration of these additional eleven markers into the high resolution mapping population will assist in the future cloning of the powdery mildew resistance genes.

Further research in our laboratory has added additional markers inside *FR1062* using AFLP (Wei *et al.* 1999). Eleven YACs have been identified from the primers of *FR1062*, *FW108* (AFLP) and *B6* (an R-gene analog) from two independent YAC library screenings. The STS primers of *FR1062* identified five of these YACs. YACs ends were used as probes mapping the position of the YACs and identifying various BACs. The identification of the BAC ends and their position on the high-resolution barley map is presently continuing to obtain a contig that spans the *Mla* resistance gene cluster (Wei *et al.* 1999). Because of the addition of new AFLP, YAC, and BAC markers, the barley map of chromosome 5 is continuously evolving.

Bibliography

Büschges, R., K. Hollricher, R. Panstruga, G. Simons, M. Wolter, A. Frijters, R. van Daelen, J. Groenendijk, S. Topsch, P. Vos, F. Salamini and P. Schulze-Lefert. (1997). The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695-705.

Bush, A. L., R. P. Wise, P. J. Rayapati, and M. Lee. (1994). Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near-isogenic lines of hexaploid oat (*Avena sativa*). Genome 37:823-831.

DeScenzo, R. A., R. P. Wise, and M. Mahadevappa. (1994). High resolution mapping of the *Hor1/Mla/Hor2* region on chromosome 5S in barley. Molecular Plant-Microbe Interactions 7:657-665.

Giovannoni, J. J., R. A. Wing, M. W. Ganal, and S. D. Tanksley. (1991). Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acid Research 19:6553-6558.

Görg, R., K. Hollricher, and P. Schulze-Lefert. (1993). Functional analysis and RFLP-mediated mapping of the *Mlg* resistance locus in barley. The Plant Journal 3(6):857-866.

Hengen, P. N. (1995). Reamplification of PCR fragments. Trends in Biochem. Sci. 20:123-124.

Hinze, K., R. D. Thompson, E. Ritter, F. Salamini, and P. Schulze-Lefert. (1991). Restriction fragment length polymorphism-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*). Proc. Natl. Acad. Sci. 88:3691-3695.

Jørgensen, J. H. (1994). Genetics of powdery mildew resistance in barley. Critical Reviews in Plant Sciences 13:97-119.

Jørgensen, J. H. and H. P. Jensen. (1976). New linkage data with the *ml-o* locus on barley chromosome 4. Barley Genet. Newsletter 6:39-41.

Jørgensen, J. H. and J. G. Moseman. (1972). Recombination at the *Mla* locus in barley conditioning resistance to *Erysiphe graminis* f. sp. *hordei*. Can. J. Genet. Cytol. 14:43-48.

Kintzios, S., A. Jahoor, and G. Fischbeck. (1995). Powdery-mildew-resistance genes *Mla29* and *Mla32* in *H. spontaneum* derived winter-barley lines. Plant Breeding 114:265-266.

Klein-Lankhorst, R. M., A. Vermont, R. Weide, T. Liharska, and P. Zabel. (1991). Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Appl. Genet. 83:108-114.

Kleine, M., W. Michalek, A. Graner, R. G. Herrmann, and C. Jung. (1993). Construction of barley (*Hordeum vulgare* L.) YAC library and isolation of a *Hor1*-specific clone. Mol. Gen. Genet. 240:265-272.

Kleine, M., W. Michalek, T. Diefenthal, H. Dargatz, and C. Jung. (1997). Construction of a *Mlu*I-YAC library from barley (*Hordeum vulgare* L.) and analysis of YAC insert terminal regions. Genome 40:896-902.

Lander, E., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newman. (1987). MAPMAKER: An Interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Mahadevappa, M., R. A. DeScenzo, and R. P. Wise. (1994). Recombination of alleles conferring specific resistance to powdery mildew at the *Mla* locus in barley. Genome 37:460-468.

Michelmore, R. W., I. Paran, and R. Kesseli. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segreganting populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Penner, G. A., J. Chong, M. Lévesque-Lemay, S. J. Molnar, and G. Fedak. (1993). Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor. Appl. Genet. 85:702-705.

Schachermayr, G., H. Siedler, M. D. Gale, H. Winzeler, M. Winzeler, and B. Keller. (1994). Identification and localization of molecular markers linked to the *Lr9* leaf rust resistance gene of wheat. Theor. Appl. Genet. 88:110-115.

Wei, F., K. Gobelman-Werner, S. M. Morroll, J. Kurth, L. Mao, R. Wing, D. Leister, P. Schulze-Lefert, and R. P. Wise. (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929-1948.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingley. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Williamson, V. M., J.-Y. Ho, F. F. Wu, N. Miller, and I. Kaloshian. (1994). A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor. Appl. Genet. 87:757-763.

Wise, R. P. and A. H. Ellingboe. (1983). Infection kinetics of *Erysiphe graminis* f. sp. *hordei* on barley with different alleles at the *Ml-a* locus. Phytopathology 73:1220-1222.

Wise, R. P. and P. S. Schnable. (1994). Mapping complementary genes in maize: positioning the *rf1* and *rf2* nuclear-fertility restorer loci of Texas (T) cytoplasm relative to RFLP and visible markers. Theor. Appl. Genet. 88:785-795.

Yu Y., J. P. Tomkins, R. Waugh, D. A. Frisch, D. Kudrna, A. Kleinhofs, R.S. Brueggeman, G. J. Muehlbauer, R. P. Wise, and R. A. Wing. (2000). A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theor. Appl. Genet. (in press).

APPENDIX A: RAPD PRIMER DATA

This appendix contains all of the data obtained using RAPD primers in a table format. The table includes the name of the primer, the quantity of fragments generated in the pools, and the marker designation.

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
OPA1	RAPD	4	4								
OPA2	RAPD	9	9								
OPA3	RAPD	12	12								
OPA4	RAPD	3	3	+	2	3	+				
OPA5	RAPD	4	3	+	4	4					
OPA6	RAPD	0	0								
OPA7	RAPD	4	4								
OPA8	RAPD	5	5								
OPA9	RAPD	1	1								
OPA10	RAPD	5	4	+	6	5	+	+		1500	$OPA10_{1500}$
OPA11	RAPD	6	6								
OPA12	RAPD	0	0								
OPA13	RAPD	7	8	+	5	5					
OPA14	RAPD	5	5								
OPA15	RAPD	1	1								
OPA16	RAPD	5	5								
OPA17	RAPD	0	0								
OPA18	RAPD	3	4	+	1	2	+				
OPA19	RAPD	8	6	+	5	6	+				
OPA20	RAPD	0	0								
OPB1	RAPD	0	4	+	4	4					
OPB2	RAPD	0	0								
OPB3	RAPD	0	0								
OPB4	RAPD	2	3	+							
OPB5	RAPD	5	5								
OPB6	RAPD	1	1								
OPB7	RAPD	7	6	+	4	3	+				
OPB8	RAPD	0	2	+	2	2					
OPB9	RAPD	0	0								
OPB10	RAPD	6	5	+	4	3	+				
OPB11	RAPD	1	2	+							
OPB12	RAPD	6	6								
OPB13	RAPD	0	0								
OPB14	RAPD	0	0								
OPB15	RAPD	6	6		6	6					
OPB16	RAPD	5	5								
OPB17	RAPD	2	2								
OPB18	RAPD	0	0								
OPB19	RAPD	0	0								
OPB20	RAPD	0	0								
UBC101	RAPD	0	1								
UBC102	RAPD	2	2								
UBC103	RAPD	3	3								
UBC104	RAPD	6	7	+	5	5					
UBC105	RAPD	0	4	+	3	3					
UBC106	RAPD	5	5		0	0					
UBC107	RAPD	0	0								
UBC108	RAPD	9	8	+	2	4	+				
UBC109	RAPD	3	3								
UBC110	RAPD	0	0								
UBC111	RAPD	1	1								
UBC112	RAPD	1	1								
UBC113	RAPD	0	0								
UBC114	RAPD	1	1								
UBC115	RAPD	1	1								
UBC116	RAPD	0	0								
UBC117	RAPD	0	0								
UBC118	RAPD	0	0								
UBC119	RAPD	2	2								
UBC120	RAPD	0	0								
UBC121	RAPD	0	0								
UBC122	RAPD	5	6	+	12	12					
UBC123	RAPD	0	0								
UBC124	RAPD	0	0								
UBC125	RAPD	9	8	+	7	7					
UBC126	RAPD	3	3								
UBC127	RAPD	4	4								
UBC128	RAPD	0	0								
UBC129	RAPD	2	2								
UBC130	RAPD	0	0								
UBC131	RAPD	3	4	+	0	4	+				
UBC132	RAPD	3	2	+	4	4					
UBC133	RAPD	4	4								
UBC134	RAPD	6	5	+	6	6					
UBC135	RAPD	5	5								
UBC136	RAPD	0	0								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC137	RAPD	0	0								
UBC138	RAPD	2	3	+	2	2					
UBC139	RAPD	0	0								
UBC140	RAPD	0	0								
UBC141	RAPD	0	1	+	0	1	+				
UBC142	RAPD	0	0								
UBC143	RAPD	1	0	+	1	1					
UBC144	RAPD	1	1								
UBC145	RAPD	5	5		6	6					
UBC146	RAPD	3	3		4	4					
UBC147	RAPD	7	8	+	12	10	+				
UBC148	RAPD	2	2								
UBC149	RAPD	6	6								
UBC150	RAPD	2	2	+	5	4	+				
UBC151	RAPD	2	3	+	2	2					
UBC152	RAPD	8	8								
UBC153	RAPD	6	10	+	4	5	+				
UBC154	RAPD	2	2								
UBC155	RAPD	11	11								
UBC156	RAPD	5	5								
UBC157	RAPD	7	7								
UBC158	RAPD	0	0								
UBC159	RAPD	8	7	+	10	11	+				
UBC160	RAPD	5	5								
UBC161	RAPD	0	0								
UBC162	RAPD	1	1								
UBC163	RAPD	1	0	+	0	0					
UBC164	RAPD	5	6	+	1	1					
UBC165	RAPD	8	0	+	11	10	+	+	+	1626	$UBC165_{1626}$
UBC166	RAPD	0	0								
UBC167	RAPD	3	3								
UBC168	RAPD	4	6	+	5	5					
UBC169	RAPD	1	2	+	0	10	+				
UBC170	RAPD	3	0	+							
UBC171	RAPD	6	6								
UBC172	RAPD	0	2	+	2	2					
UBC173	RAPD	EMPTY[h]									
UBC174	RAPD	7	7								
UBC175	RAPD	5	5	+							
UBC176	RAPD	6	5	+	4	1	+				
UBC177	RAPD	3	3								
UBC178	RAPD	8	8								
UBC179	RAPD	0	0								
UBC180	RAPD	13	12	+	7	8	+				
UBC181	RAPD	12	12								
UBC182	RAPD	1	1								
UBC183	RAPD	3	4	+	4	4					
UBC184	RAPD	4	4								
UBC185	RAPD	7	7								
UBC186	RAPD	6	6								
UBC187	RAPD	EMPTY									
UBC188	RAPD	9	9								
UBC189	RAPD	4	6	+	4	4					
UBC190	RAPD	5	4	+	5	5					
UBC191	RAPD	3	3								
UBC192	RAPD	8	8								
UBC193	RAPD	2	1	+	1	1					
UBC194	RAPD	7	7								
UBC195	RAPD	7	7								
UBC196	RAPD	8	8								
UBC197	RAPD	2	2	+	2	1	+				
UBC198	RAPD	5	5								
UBC199	RAPD	7	8	+	7	9	+				
UBC200	RAPD	0	0								
UBC201	RAPD	0	0								
UBC202	RAPD	8	8								
UBC203	RAPD	4	6	+	7	5	+				
UBC204	RAPD	6	6								
UBC205	RAPD	2	4	+	2	4	+				
UBC206	RAPD	5	5								
UBC207	RAPD	0	0								
UBC208	RAPD	9	9								
UBC209	RAPD	2	2								
UBC210	RAPD	5	5								
UBC211	RAPD	12	8	+	6	6					
UBC212	RAPD	5	6	+	3	3					
UBC213	RAPD	6	6								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC214	RAPD	1	0	+	2	2					
UBC215	RAPD	6	6								
UBC216	RAPD	0	0								
UBC217	RAPD	0	0								
UBC218	RAPD	7	8	+	7	7					
UBC219	RAPD	7	7								
UBC220	RAPD	2	2								
UBC221	RAPD	3	4	+	5	5					
UBC222	RAPD	6	6								
UBC223	RAPD	9	9								
UBC224	RAPD	0	0								
UBC225	RAPD	7	7								
UBC226	RAPD	5	5								
UBC227	RAPD	4	0	+	6	6					
UBC228	RAPD	8	8								
UBC229	RAPD	0	3	+	4	3	+				
UBC230	RAPD	11	11		4	4					
UBC231	RAPD	13	10	+	8	7	+				
UBC232	RAPD	10	10								
UBC233	RAPD	0	0								
UBC234	RAPD	7	3	+	7	7					
UBC235	RAPD	3	3								
UBC236	RAPD	7	5	+	7	8	+				
UBC237	RAPD	9	9								
UBC238	RAPD	8	0	+	6	6					
UBC239	RAPD	6	5	+	4	6	+				
UBC240	RAPD	0	2	+	3	3					
UBC241	RAPD	7	7								
UBC242	RAPD	0	6	+	6	6					
UBC243	RAPD	6	12	+	7	7					
UBC244	RAPD	5	13	+	7	9	+				
UBC245	RAPD	12	12								
UBC246	RAPD	2	2								
UBC247	RAPD	3	3								
UBC248	RAPD	11	11								
UBC249	RAPD	0	7	+	4	5	+				
UBC250	RAPD	9	0	+	10	10					
UBC251	RAPD	5	5								
UBC252	RAPD	1	5	+	3	3					
UBC253	RAPD	8	8								
UBC254	RAPD	6	6								
UBC255	RAPD	0	0								
UBC256	RAPD	6	6								
UBC257	RAPD	2	2								
UBC258	RAPD	0	5	+	5	5					
UBC259	RAPD	2	5	+	5	6	+				
UBC260	RAPD	0	0								
UBC261	RAPD	8	8								
UBC262	RAPD	7	7								
UBC263	RAPD	0	0								
UBC264	RAPD	4	4								
UBC265	RAPD	6	6								
UBC266	RAPD	10	10								
UBC267	RAPD	1	1								
UBC268	RAPD	7	7								
UBC269	RAPD	0	7	+	7	7					
UBC270	RAPD	9	9								
UBC271	RAPD	2	2								
UBC272	RAPD	11	11								
UBC273	RAPD	7	7								
UBC274	RAPD	5	5								
UBC275	RAPD	4	4								
UBC276	RAPD	7	7								
UBC277	RAPD	5	5								
UBC278	RAPD	4	4								
UBC279	RAPD	0	0								
UBC280	RAPD	10	10								
UBC281	RAPD	4	4								
UBC282	RAPD	2	2								
UBC283	RAPD	7	6	+	7	9	+				
UBC284	RAPD	14	14								
UBC285	RAPD	14	14								
UBC286	RAPD	0	0								
UBC287	RAPD	6	5	+	8	8					
UBC288	RAPD	1	2	+	2	2					
UBC289	RAPD	0	11	+	11	13	+				
UBC290	RAPD	10	10								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC291	RAPD	2	2								
UBC292	RAPD	11	11								
UBC293	RAPD	1	1								
UBC294	RAPD	0	0								
UBC295	RAPD	12	12								
UBC296	RAPD	12	16	+	13	13					
UBC297	RAPD	4	6	+	5	4	+				
UBC298	RAPD	4	5	+	3	4	+				
UBC299	RAPD	5	8	+	5	5					
UBC300	RAPD	8	9	+	11	11					
UBC301	RAPD	3	3								
UBC302	RAPD	3	0	+	5	8	+				
UBC303	RAPD	3	3								
UBC304	RAPD	0	0								
UBC305	RAPD	0	0								
UBC306	RAPD	1	0	+	1	0	+				
UBC307	RAPD	0	0								
UBC308	RAPD	8	8								
UBC309	RAPD	1	1								
UBC310	RAPD	1	2	+	1	3	+				
UBC311	RAPD	0	0								
UBC312	RAPD	10	10								
UBC313	RAPD	3	4	+	3	3					
UBC314	RAPD	0	0								
UBC315	RAPD	4	5	+	4	5	+				
UBC316	RAPD	2	0	+	2	0	+				
UBC317	RAPD	0	0								
UBC318	RAPD	3	3								
UBC319	RAPD	0	0								
UBC320	RAPD	2	0	+	1	4	+				
UBC321	RAPD	0	0								
UBC322	RAPD	6	6								
UBC323	RAPD	3	2	+	3	3					
UBC324	RAPD	6	1	+	6	3	+				
UBC325	RAPD	0	0								
UBC326	RAPD	0	0								
UBC327	RAPD	3	3								
UBC328	RAPD	0	0								
UBC329	RAPD	8	11	+	11	16	+				
UBC330	RAPD	0	0								
UBC331	RAPD	2	0	+	2	1	+				
UBC332	RAPD	0	0								
UBC333	RAPD	1	1								
UBC334	RAPD	0	0								
UBC335	RAPD	6	6								
UBC336	RAPD	7	7								
UBC337	RAPD	2	2								
UBC338	RAPD	6	6								
UBC339	RAPD	0	0								
UBC340	RAPD	11	11								
UBC341	RAPD	1	1								
UBC342	RAPD	4	4								
UBC343	RAPD	0	0								
UBC344	RAPD	0	0								
UBC345	RAPD	8	8								
UBC346	RAPD	3	3								
UBC347	RAPD	0	0								
UBC348	RAPD	6	0	+							
UBC349	RAPD	0	0								
UBC350	RAPD	6	2	+	6	6					
UBC351	RAPD	3	0	+	4	4					
UBC352	RAPD	0	0								
UBC353	RAPD	10	1	+	10	10					
UBC354	RAPD	10	0	+	10	10					
UBC355	RAPD	0	2	+							
UBC356	RAPD	10	0	+	12	12					
UBC357	RAPD	6	12	+	7	8	+				
UBC358	RAPD	6	8	+	6	5	+				
UBC359	RAPD	9	4	+	7	6	+				
UBC360	RAPD	0	6	+							
UBC361	RAPD	10	9	+	6	6					
UBC362	RAPD	6	0	+							
UBC363	RAPD	0	0								
UBC364	RAPD	4	0	+	4	4					
UBC365	RAPD	1	0	+	1	1					
UBC366	RAPD	0	0								
UBC367	RAPD	0	0								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC368	RAPD	0	0								
UBC369	RAPD	0	0								
UBC370	RAPD	8	7	+	4	4					
UBC371	RAPD	0	0								
UBC372	RAPD	8	0	+	4	7	+				
UBC373	RAPD	9	8	+							
UBC374	RAPD	10	16	+	9	8	+				
UBC375	RAPD	8	0	+	9	9					
UBC376	RAPD	9	9								
UBC377	RAPD	0	0								
UBC378	RAPD	0	2	+	2	2					
UBC379	RAPD	9	9								
UBC380	RAPD	2	2								
UBC381	RAPD	0	4	+	4	3	+				
UBC382	RAPD	2	4	+	4	5	+				
UBC383	RAPD	6	7	+	7	7					
UBC384	RAPD	0	0								
UBC385	RAPD	3	0	+	6	8	+				
UBC386	RAPD	4	0	+							
UBC387	RAPD	7	3	+	4	4					
UBC388	RAPD	6	6								
UBC389	RAPD	11	0	+	13	13					
UBC390	RAPD	0	0								
UBC391	RAPD	8	8								
UBC392	RAPD	1	0	+	1	1					
UBC393	RAPD	2	2								
UBC394	RAPD	0	0								
UBC395	RAPD	2	0	+	2	2					
UBC396	RAPD	6	7	+	12	12					
UBC397	RAPD	0	0								
UBC398	RAPD	0	0								
UBC399	RAPD	5	4	+	4	2	+				
UBC400	RAPD	0	0								
UBC401	RAPD	0	0								
UBC402	RAPD	0	0								
UBC403	RAPD	4	3	+	3	3					
UBC404	RAPD	0	0								
UBC405	RAPD	0	0								
UBC406	RAPD	0	0								
UBC407	RAPD	3	3								
UBC408	RAPD	0	0								
UBC409	RAPD	10	11	+	7	7					
UBC410	RAPD	0	0								
UBC411	RAPD	5	6	+	4	8	+				
UBC412	RAPD	4	2	+	4	5	+				
UBC413	RAPD	9	8	+	12	12					
UBC414	RAPD	8	1	+	7	7					
UBC415	RAPD	0	0								
UBC416	RAPD	0	0								
UBC417	RAPD	0	0								
UBC418	RAPD	13	10	+	9	9					
UBC419	RAPD	0	0								
UBC420	RAPD	7	0	+	7	8	+				
UBC421	RAPD	0	0								
UBC422	RAPD	0	0								
UBC423	RAPD	6	5	+	8	6	+				
UBC424	RAPD	9	9								
UBC425	RAPD	8	5	+	5	6	+				
UBC426	RAPD	0	0								
UBC427	RAPD	0	0								
UBC428	RAPD	0	0								
UBC429	RAPD	0	0								
UBC430	RAPD	0	0								
UBC431	RAPD	10	10								
UBC432	RAPD	11	8	+	8	8					
UBC433	RAPD	0	0								
UBC434	RAPD	0	0								
UBC435	RAPD	0	0								
UBC436	RAPD	2	2								
UBC437	RAPD	1	0	+	1	1					
UBC438	RAPD	2	2								
UBC439	RAPD	0	0								
UBC440	RAPD	0	0								
UBC441	RAPD	0	0								
UBC442	RAPD	0	0								
UBC443	RAPD	0	0								
UBC444	RAPD	5	5								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC445	RAPD	3	7	+	5	4	+				
UBC446	RAPD	2	2								
UBC447	RAPD	0	0								
UBC448	RAPD	6	6								
UBC449	RAPD	5	0	+	5	4	+				
UBC450	RAPD	6	6								
UBC451	RAPD	0	0								
UBC452	RAPD	0	0								
UBC453	RAPD	0	0								
UBC454	RAPD	0	0								
UBC455	RAPD	6	6								
UBC456	RAPD	7	7		7	7					
UBC457	RAPD	2	0	+	4	4					
UBC458	RAPD	0	0								
UBC459	RAPD	9	7	+	10	10					
UBC460	RAPD	0	0								
UBC461	RAPD	0	0								
UBC462	RAPD	0	1	+	6	7	+				
UBC463	RAPD	0	0								
UBC464	RAPD	2	2								
UBC465	RAPD	10	7	+	9	7	+	+	+	950	$UBC465_{950}$
UBC466	RAPD	0	0								
UBC467	RAPD	11	9	+	11	10	+				
UBC468	RAPD	8	10	+	14	14					
UBC469	RAPD	0	0								
UBC470	RAPD	5	4	+	4	4					
UBC471	RAPD	4	3	+	8	8					
UBC472	RAPD	6	5	+	6	6					
UBC473	RAPD	0	0								
UBC474	RAPD	8	7	+	10	10					
UBC475	RAPD	1	0	+	1	1					
UBC476	RAPD	3	3								
UBC477	RAPD	4	4								
UBC478	RAPD	0	0								
UBC479	RAPD	0	0								
UBC480	RAPD	3	2	+	3	3					
UBC481	RAPD	0	0		2	2					
UBC482	RAPD	0	0								
UBC483	RAPD	0	0								
UBC484	RAPD	0	0								
UBC485	RAPD	4	5	+	2	7	+				
UBC486	RAPD	0	0								
UBC487	RAPD	5	6	+	10	14	+				
UBC488	RAPD	0	0								
UBC489	RAPD	10	10								
UBC490	RAPD	0	0								
UBC491	RAPD	0	0								
UBC492	RAPD	0	0								
UBC493	RAPD	0	0								
UBC494	RAPD	0	0								
UBC495	RAPD	0	0								
UBC496	RAPD	0	0								
UBC497	RAPD	3	3								
UBC498	RAPD	1	1								
UBC499	RAPD	8	8								
UBC500	RAPD	0	0								
UBC501	RAPD	0	0								
UBC502	RAPD	0	0								
UBC503	RAPD	0	0								
UBC504	RAPD	0	0								
UBC505	RAPD	0	0								
UBC506	RAPD	0	0								
UBC507	RAPD	0	0								
UBC508	RAPD	5	6	+	6	7	+				
UBC509	RAPD	0	0								
UBC510	RAPD	0	0								
UBC511	RAPD	6	6								
UBC512	RAPD	9	9								
UBC513	RAPD	0	6	+	6	6					
UBC514	RAPD	1	3	+	0	1	+				
UBC515	RAPD	0	0								
UBC516	RAPD	8	8								
UBC517	RAPD	6	8	+	7	7					
UBC518	RAPD	2	3	+	3	3					
UBC519	RAPD	0	0								
UBC520	RAPD	0	0								
UBC521	RAPD	10	0	+	10	10					

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC522	RAPD	0	0								
UBC523	RAPD	3	9	+	9	9					
UBC524	RAPD	0	0								
UBC525	RAPD	0	0								
UBC526	RAPD	5	5								
UBC527	RAPD	0	0								
UBC528	RAPD	6	6	+	6	6	+				
UBC529	RAPD	0	0								
UBC530	RAPD	0	0								
UBC531	RAPD	0	0								
UBC532	RAPD	3	3	+	3	3	+				
UBC533	RAPD	0	0								
UBC534	RAPD	2	3	+	2	4	+				
UBC535	RAPD	0	0								
UBC536	RAPD	0	0								
UBC537	RAPD	4	7	+	12	12					
UBC538	AFLP	8	8								
UBC539	AFLP	0	0								
UBC540	AFLP	18	18								
UBC541	AFLP	0	0								
UBC542	AFLP	0	0								
UBC543	AFLP	7	7								
UBC544	AFLP	1	3	+	9	9					
UBC545	AFLP	0	0								
UBC546	AFLP	0	0								
UBC547	AFLP	0	0								
UBC548	AFLP	14	14								
UBC549	AFLP	0	0								
UBC550	AFLP	7	6	+	9	7	+				
UBC551	AFLP	0	0								
UBC552	AFLP	6	6	+	3	4	+				
UBC553	AFLP	1	1								
UBC554	AFLP	0	0								
UBC555	AFLP	9	12	+	12	12					
UBC556	AFLP	8	7	+	9	9					
UBC557	AFLP	6	6								
UBC558	AFLP	0	0								
UBC559	AFLP	9	9								
UBC560	AFLP	0	0								
UBC561	AFLP	0	0								
UBC562	AFLP	4	7	+	7	7					
UBC563	AFLP	0	0								
UBC564	AFLP	10	12	+	8	10	+				
UBC565	AFLP	0	0								
UBC566	AFLP	5	5								
UBC567	AFLP	0	0								
UBC568	AFLP	0	0								
UBC569	AFLP	6	6								
UBC570	AFLP	4	5	+	4	5	+				
UBC571	AFLP	5	5								
UBC572	AFLP	0	0								
UBC573	AFLP	0	0								
UBC574	AFLP	14	14								
UBC575	AFLP	10	12	+	10	10					
UBC576	AFLP	0	0								
UBC577	AFLP	0	0								
UBC578	AFLP	5	5								
UBC579	AFLP	6	3	+	5	8	+				
UBC580	AFLP	7	7								
UBC581	AFLP	0	0								
UBC582	AFLP	0	0								
UBC583	AFLP	0	0								
UBC584	AFLP	14	14								
UBC585	AFLP	8	6	+	5	5					
UBC586	AFLP	8	7	+	8	9	+				
UBC587	AFLP	0	0								
UBC588	AFLP	10	9	+	13	13					
UBC589	AFLP	10	9	+	10	11	+				
UBC590	AFLP	2	2								
UBC591	AFLP	0	0								
UBC592	AFLP	1	10	+							
UBC593	AFLP	2	1	+	7	7					
UBC594	AFLP	8	6	+	8	8					
UBC595	AFLP	5	7	+	4	6	+				
UBC596	AFLP	3	1	+	0	3	+				
UBC597	AFLP	0	0								
UBC598	AFLP	6	6								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC599	AFLP	0	0								
UBC600	AFLP	0	0								
UBC601	AFLP	5	6	+	6	6					
UBC602	AFLP	4	4								
UBC603	AFLP	0	0								
UBC604	AFLP	6	5	+	5	5					
UBC605	AFLP	0	0								
UBC606	AFLP	12	12								
UBC607	AFLP	8	7	+	5	7	+				
UBC608	AFLP	7	7								
UBC609	AFLP	11	11								
UBC610	AFLP	7	7								
UBC611	AFLP	3	3								
UBC612	AFLP	4	4								
UBC613	AFLP	8	7	+	11	11					
UBC614	AFLP	4	4	+							
UBC615	AFLP	11	12	+	15	15					
UBC616	AFLP	7	0	+	7	7					
UBC617	AFLP	1	6	+	6	6					
UBC618	AFLP	1	2	+	3	3					
UBC619	AFLP	0	0								
UBC620	AFLP	5	5								
UBC621	AFLP	0	0								
UBC622	AFLP	5	3	+	5	5					
UBC623	AFLP	13	9	+	14	14					
UBC624	AFLP	0	5	+	10	9	+				
UBC625	AFLP	7	7								
UBC626	AFLP	4	4								
UBC627	AFLP	0	0								
UBC628	AFLP	0	0								
UBC629	AFLP	0	0								
UBC630	AFLP	0	0								
UBC631	AFLP	3	3								
UBC632	AFLP	0	6	+	15	15					
UBC633	AFLP	0	1	+	5	5					
UBC634	AFLP	10	10	+							
UBC635	AFLP	7	11	+	16	15	+				
UBC636	AFLP	5	9	+	6	6					
UBC637	AFLP	2	4	+	6	6					
UBC638	AFLP	0	10	+	14	14					
UBC639	AFLP	11	11	+							
UBC640	AFLP	8	8	+							
UBC641	AFLP	14	14	+							
UBC642	AFLP	10	10	+							
UBC643	AFLP	9	9	+							
UBC644	AFLP	0	0								
UBC645	AFLP	1	1								
UBC646	AFLP	6	6								
UBC647	AFLP	0	0								
UBC648	AFLP	6	6								
UBC649	AFLP	12	10	+	12	12					
UBC650	AFLP	3	3								
UBC651	AFLP	7	6	+	7	7					
UBC652	AFLP	11	11								
UBC653	AFLP	0	0								
UBC654	AFLP	9	9								
UBC655	AFLP	9	10	+	11	11					
UBC656	AFLP	1	1								
UBC657	AFLP	1	1								
UBC658	AFLP	0	0								
UBC659	AFLP	4	10	+	12	12					
UBC660	AFLP	14	14								
UBC661	AFLP	4	4	+							
UBC662	AFLP	13	13								
UBC663	AFLP	8	9	+	10	5	+				
UBC664	AFLP	6	6								
UBC665	AFLP	0	0								
UBC666	AFLP	4	5	+							
UBC667	AFLP	5	5								
UBC668	AFLP	4	4								
UBC669	AFLP	0	0								
UBC670	AFLP	3	4	+	5	8	+				
UBC671	AFLP	10	12	+	14	10	+				
UBC672	AFLP	2	2								
UBC673	AFLP	2	5	+	6	6					
UBC674	AFLP	8	8		11	11					
UBC675	AFLP	13	13								

	Pools[b]	Number of Bands Amplified (Initial)			Number of Bands (Final)			High Resolution Mapping Population[f]			
RAPD Primer[a]	RAPD or AFLP Pools	Pool A[c]	Pool B[d]	PM[e]	Pool A[c]	Pool B[d]	PM	RIMP	COSEG	Size bp	Marker Designation[g]
UBC676	AFLP	10	6	+	11	8	+				
UBC677	AFLP	5	5	+	3	3					
UBC678	AFLP	0	0								
UBC679	AFLP	10	10								
UBC680	AFLP	5	5								
UBC681	AFLP	7	18	+	10	10					
UBC682	AFLP	16	16	+							
UBC683	AFLP	0	0								
UBC684	AFLP	2	8	+	8	11	+				
UBC685	AFLP	11	11		9	9					
UBC686	AFLP	8	8	+							
UBC687	AFLP	0	0								
UBC688	AFLP	11	11								
UBC689	AFLP	2	6	+	1	7	+				
UBC690	AFLP	1	1								
UBC691	AFLP	9	10	+	8	8					
UBC692	AFLP	0	0								
UBC693	AFLP	4	4								
UBC694	AFLP	11	11								
UBC695	AFLP	0	0								
UBC696	AFLP	9	9								
UBC697	AFLP	11	11								
UBC698	AFLP	3	3								
UBC699	AFLP	7	7								
UBC700	AFLP	0	0								
TOTAL		2400	2399								

[a]RAPD primers from Operon Technologies Inc., Alameda, CA (OP) and Oligonucleotide Synthesis Laboratory, University British Columbia, Vancouver, Canada (UBC).

[b]RAPD pools are the original pools and the AFLP pools are the adjusted pools.

[c]Pool A: The barley recombinant pool that contains the *Mla6* resistance allele.

[d] Pool B: The barley recombinant pool that contains the *Mla13* resistance allele.

[e]PM: + represents the presence or absence of a product, while represents no difference in the products amplified.

[f]High resolution - mapping populations are the two populations used to map polymorphic primers. The recombinant interval mapping population (RIMP) consists of 2 reciprocal recombination events per mapping interval between *Hor1* and *Hor2*. The fine - mapping barley population consists of 100 individuals that have one recombination event only between the X*mwg068* to X*mwg036*.

[g] Marker designation is the name given to the new markers when place on the barley map.

[h]Empty refers to a particular primer was not available.

APPENDIX B: EXPLORATORY RESEACH

This appendix contains the information gathered during the exploratory research. Preliminary steps must be accomplished and verified to achieve the objective of the research project. The following is a technical look at the procedures used in this research. This appendix includes the determination of pool size, the efficiency of bulked segregant analysis, technical protocols, and bibliography.

Determination of Size of the Pool

Bulked segregant analysis is the pooling of DNA from individuals in a segregating population. The difficulty with bulked segregant analysis is the generation of false positives due to incorrect pool size. Predetermining the size of the pool eliminates the majority of false positives (Giovannoni *et al.* 1991; Michelmore *et al.* 1991).

Individual DNAs of the parental accessions were pooled together in various dilutions. The resistance specificities *Mla6* + *Mla14*, and *Mla13* + *Ml–Ru3* are contained in C.I. 16151 and C.I. 16155, respectively. The DNA of C.I. 16151 and C.I. 16155 were pooled together in the following dilutions: 1:1, 1:2, 1:4, 1:8, 1:12, 1:16, and 1:20. Figure 5 represents the preparatory research to determine a feasible pool size. These pools were tested with four primers to reveal various polymorphisms in a particular pool. The OPA-11 primer (Fig. 5A) reveals a C.I.16151-amplified DNA product in the original parental lane and only in the 1:1 dilution. This primer requires more than two individuals in the pool to identify a polymorphism. Whereas, the OPA-10 primer (Fig. 5B) displayed a C.I. 16155-amplified DNA product in the original parental lane and a slight C.I. 16155-amplified DNA product still in the 1:8 dilution. This primer would require the pools to contain greater than eight individuals. The OPA-07 primer (Fig. 5C), alternatively, has a C.I. 16151-amplified DNA product in the original parental lane and a slight product visible up to the 1:20 dilution. The UMC150 primer (Fig. 5D) has a C.I. 16155-amplified DNA product in the original parental lane and reveals a C.I. 16155-amplified DNA product in all the dilutions. These primers would need more than sixteen individuals in a pool. These preliminary experiments imply that various pool sizes would be necessary for different RAPD primers. To eliminate the various sizes required it was determined that the pools should contain more than ten individuals but less than

twenty. Therefore, Pool-A was composed of DNA isolated from 14 individuals that were homozygous for the *Mla6* and *Mla14* specificities. Pool-B was composed of DNA isolated from 14 individuals that were homozygous for the *Mla13* and *Ml–Ru3* specificities.

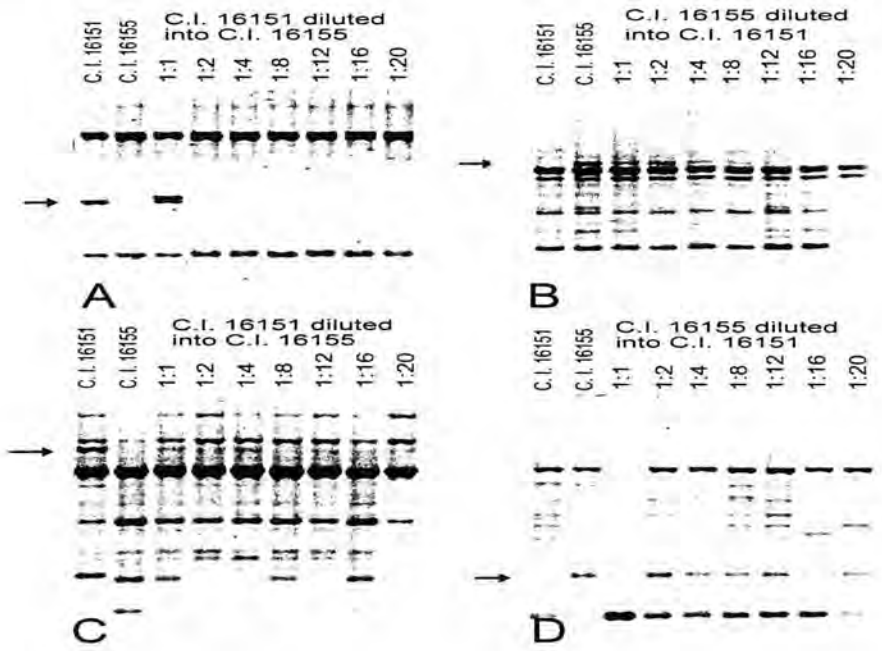

Fig. 5. Feasible pool size determination. The accession C.I. 16151 contains the resistance specificities *Mla6* + *Mla14,* while C.I. 16155 has *Mla13* + *Ml-Ru3.* The presence of DNA amplification product in the dilutions indicates the sensitivity of the pools to various primers. This helps determine the pool size and reduce the generation of false positives. The arrows indicate the presence of fragments in the barley accessions and the dilutions.

A. The DNA was amplified from C.I. 16151and in the 1:1 dilution with primer OPA-11 (Operon Technologies).

B. The DNA was amplified from C.I. 16155 with primer OPA-10 (Operon Technologies) and in all the dilutions through 1:8.

C. The DNA was amplified from C.I. 16151 with primer OPB-07 (Operon Technologies) and in all dilutions through 1:20.

D. The DNA was amplified from C.I. 16155 with primer UBC150 (Carlson) and in all the dilutions.

Efficiency of Bulked Segregant Analysis

Bulked segregant analysis allows for the identification of new markers in an area of interest. To demonstrate the proficiency of bulked segregant analysis in finding new markers in the interval of interest, the RAPD primers were tested on DNAs of both the pools and the individual barley accessions. When both the accession and the pool amplify the same size fragment then the fragment may be in the region of interest. When the primer amplifies product in the accession and not in the corresponding pool, then the detected fragment is not in the region of interest. When the primer amplifies a product in the pool and not in the accession, then the detected fragment may be in the region of interest.

Forty-eight RAPD primers were tested on C.I. 16151, C.I. 16155, Pool-A, and Pool-B. Figure 6 represents the differences between the pools and the parental accessions in the DNA products amplified. Various polymorphisms were detected in the pools and the accessions. The primer UBC523 (Fig. 6A) is an example of the accessions and pools amplifying the identical product pattern. Both the accessions and the pools are capable of identifying alike fragments. The primers UBC508 (Fig. 6B) and UBC159 (Fig. 6C) are examples of a pattern difference between the barley parental accessions and the pools. Primer UBC508 (Fig. 6B) generated a C.I. 16155-amplified DNA product, but does not appear in either pool. Primer UBC159 (Fig. 6C) identifies two different polymorphisms. One C.I. 16155-amplified DNA product is present in the accession, but is not present in either pool. This polymorphism identified is not in the region of interest. Whereas, the other C.I. 16155-amplified DNA product amplifies in both the accession and Pool-B. The polymorphism that may map to the region of interest.

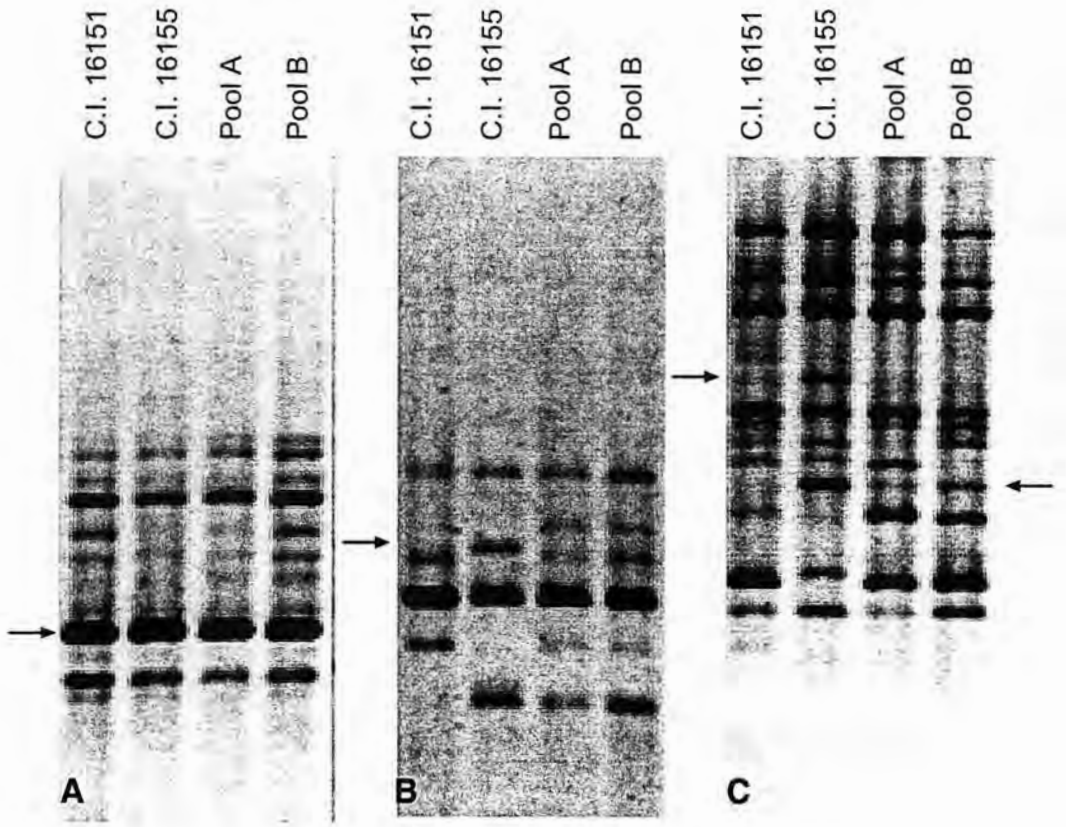

Fig. 6. Agarose gel electrophoresis demonstrating the efficiency bulked segregant analysis in comparison to the entire genome with barley accessions. The accession lines C.I. 16151 and C.I. 16155 contain the *Mla6-* and *Mla13-* resistance specificities. Pool A and B each contain genomic DNAs from 14 homozygous recombinant lines containing the *Mla6-* and *Mla13-* resistance specificities, respectively. Arrows indicate:

A. A similar DNA amplification pattern was generated in both the accessions and pools with UBC523 RAPD primer.

B. The presence of a fragment in the C.I. 16155 accession generated with UBC508 RAPD primer, which was not in either of the pools. The polymorphism may be anywhere in the entire barley genome, but not present in the interval of interest.

C. The different DNA amplification products the UBC-159 RAPD primer generated between the accessions and the pools. The top arrow indicates the presence of a DNA amplification product in the C.I. 16155 accession, which was not present in the pools. Whereas, the bottom arrow indicates the presence of a DNA amplification product in both the C.I. 16155 accession and Pool B. Similar polymorphic DNA amplification product which may indicate the RAPD primer generating a similar region.

Insert Preparation

A modified method of recovering DNA from agarose gels was used (He 1992; Li 1993; Hengen 1994). A small hole was pierced the bottom of a 0.5-ml centrifuge tube and a small piece of WHATMAN GF/C glass microfiber filters (Whatman Labsales, Hillsboro, OR) blocked the aperture. The sterile 0.5 ml centrifuge tube was placed into a sterile 1.5 ml centrifuge tube. The amplification product was excised from the agarose gel, placed in a 0.5 ml centrifuge tube, and centrifuged for 30 seconds at 15,000 rpm. The buffer solution containing the DNA was passed through the glass microfiber filter into the 1.5 ml centrifuge tube. DNA fragments were resolved by electrophoresis at 80 volts for 4 hours on a 2% thin (3 mm) agarose gel containing 1 X TBE buffer (0.089 M Tris, 0.089 M Borate, 0.002 M EDTA) (Sambrook *et al.*1989). This technique was referred to as the centrifuge-squish method. This method may also co-elute contaminants that may effect further evaluations of the DNA.

An alternate method for excising, purifying, and recovering large percentage of the nucleic acid was to use a modified S&S procedure requiring NA45 paper (Schleicher & Schuell, Keene, NH). The DNA of interest was resolved by electrophoresis to achieve good separation. The interception of the nucleic acid DNA fragment was achieved by inserting NA45 paper below the nucleic acid DNA fragment. Gel electrophoresis was resumed until the nucleic acid was bound to the NA45 paper. The paper was removed from the gel with forceps and placed in a sterile 1.5 ml centrifuge tube containing 300 µl High Salt (1.0 M NaCl, 0.1 mM Na_2EDTA, 20 mM Tris-HCl, pH 8) to extract the nucleic acid from the NA45 paper. The tube was incubated for 30 - 60 minutes at 65°C, agitating every 15 minutes. The High Salt solution was removed and placed into a new sterile 1.5-ml centrifuge tube. A phenol-chloroform extraction (75% phenol:25% chloroform:isoamyl alcohol)

was performed twice on the high salt solution. The nucleic acid was precipitated, washed, and re-suspended in the appropriate volume of distilled water. The DNA concentration was verified by either gel electrophoresis or GeneQuant II (Pharmacia, Piscataway, NJ).

Bibliography

Giovannoni, J. J., R. A. Wing, M. W. Ganal, and S. D. Tanksley. (1991). Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acid Research 19:6553-6558.

He, M., H. Liu, Y. Wang, and B. Austen. (1992). Optimized centrifugation for rapid elution of DNA from agarose gels. Genet. Anal. Tech. Appl. 9(1):31-33.

Hengen, P. N. (1994). Recovering DNA from agarose gels. Trends in Biochem. Sci. 19:388-389.

Li, Quanzhi and C. L. Ownby. (1993). A rapid method for extraction of DNA from agarose gels using a syringe. BioTechniques 15(6):976-978.

Michelmore, R. W., I. Paran, and R. Kesseli. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segreganting populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.

Sambrook, J., E.F. Fritsch, and T Maniatis. (1989). Molecular cloning : a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, 3 v. Cold Spring Harbor, N.Y.

APPENDIX C: ADDITONAL REFERENCES FOR THE LABORATORY

This appendix includes additional references for setting up PCR and other useful references.

Allard, M. W., D. L. Ellsworth, and R. L. Honeycutt. (1991). The production of single-stranded DNA suitable for sequencing using the polymerase chain reaction. BioTechniques 10(1):24-26.

Anonymous. (1990). Elimination of PCR Contamination with UV light. FOTO Dynamics. 6:1-2.

Barany, F. (1991). Genetic disease detection and DNA amplification using cloned thermostable ligase. Proc. Natl. Acad. Sci. USA Genetics 88:189-193.

Bartoli, F., M. Nuzzo, M. Pezzanera, A. Nicosia, and P. Monaci. (1996). Rapid protocol for template preparation of large number of clones. BioTechniques 20(4):554-558.

Bechhofer, D. H. (1991). A method for sequencing polymerase chain reaction products can be used to sequence *Bacillus subtilis* miniprep plasmid DNA. BioTechniques 10(1):17-20.

Bhat, G. J., M. J. Lodes, P. J. Myler and K. D. Stuart. (1990). A simple method for cloning blunt ended DNA fragments. Nucleic Acids Research 19(2):398.

Boleda, M. D., P. Briones, J. Farrés, L. Tyfield, and R. Pi. (1996). Experimental design: A useful tool for PCR optimization. BioTechniques 21(1):134-140.

Bottema, C. D. K., G. Sarkar, J. D. Cassady, S. Li, C. M. Dutton, and S. S. Sommer. (1993). Polymerase chain reaction amplification of specific alleles: A general method of detection of mutations, polymorphisms, and haplotypes. Methods on Enzymology, Academic Press, Inc. 218:388-402.

Corley-Smith, G. E., C. J. Lim, G. B. Kalmar, and B. P. Brndhorst. (1997). Efficient detection of DNA polymorphisms by fluorescent RAPD analysis. BioTechniques 22(4):690-699.

Church, G. M., and W. Gilbert. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA. 81:1991-1995.

DeFlippers. (1991). Decontaminating the polymerase chain reaction. BioTechniques 10(1):26.

Dionne, C. A. and M. Jaye. (1993). Chromosome assignment by polymerase chain reaction technique. Methods in enzymology, Academic Press, Inc. 218:402-413.

Dorit, R. L., O. OHara, and W. Gilbert. (1993). One-sided anchored Polymerase Chain Reaction for Amplification and sequencing of complementary DNA. Methods in Enzymology. 218:36-47.

Du, Z., L. Hood, and R. K. Wilson. (1993). Automated fluorscent DNA sequencing of polymerase chain reaction products. Methods in Enzymology. 218:105-121.

Feinberg, A. P., and B. Vogelstein. (1983). A technique for radiolabelling DNA fragments to high specific activity. Anal. Biochem. 132:6-32.

Gold, P. (1992). Use of a novel agarose gel-digesting enyzme for easy and rapid purification of PCR-amplified DNA for sequencing. Biotechniques 13(1):132-134.

Hengen, P. N. (1994). Recovering DNA from agarose gels. Trends in Biochem. Sci. 19:388-389.

Hengen, P. N. (1995). Reamplification of PCR fragments. Trends in Biochem. Sci 20:123-124.

Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. (1990). PCR Protocols: A guide to methods and applications. San Diego, CA, Academic Press, Inc.

Joshi, A. K., V. Baichwal, and G. F.-L. Ames. (1991). Rapid polymerase chain reaction amplification using intact bacteral cells. BioTechniques 10(1):42-43.

Kadokami, Y. and R. V. Lewis. (1994). Repeated PCR of a gel band can be used to obtain a single PCR band. Biotechniques 17(3):438.

Karuppiah, N. and P. B. Kaufman. (1992). Rapid and inexpensive micro-electroelution of nucleic acid and protein from agarose and polyacrylamide gels. Biotechniques 13(3):368.

Kavalic, D., J.-H. Kwak, and B. Weisblum. (1991). General method for direct cloning of DNA fragments generated by the polymerase chain reaction. Nucleic Acid Research 19(16):4520.

Khristich, J. V., J. Bailis, K. Diggle, A. Rodkins, A. Romo, J. Quackenbush, and G.A. Evans. (1994). Large-scale screeening of yeast artificial chromosome libraries using PCR. BioTechniques 17(3):498-501.

Kretz, K. A. and J. S. O'Brien. (1993). Direct sequencing of polymerase chain reactioin products from low melting temperature agarose. Methods in Enzymology. 218:72-79.

Kwok, S. and R. Higuchi. (1989). Avoiding false positives with PCR. Nature 339:237.

Landweber, L. F. and M. Kreitman. (1993). Producing single-stranded DNA in Polymerase Chain reaction for direct genomic sequencing. Methods of Enzymology. 218:17-26.

Lawrence, J. G., D. L. Hartl, and H. Ochman. (1993). Sequencing products of Polymerase Chain Reaction. Method of Enzymology. 218:26-35.

Lee, A. B. and T. A. Cooper. (1995). Improved direct PCR screen for bacterial colonies: Wooden toothpicks inhibit PCR amplification. BioTechniques 18(2):225-226.

Liu, Z. and P. B. Hackett. (1993). Reverse cloning procedure for generation of subclones for DNA sequencing. Methods of Enzymology. 218:47-59.

Liu, Z.-g. and L. M. Schwartz. (1992). An efficient method for blunt-end ligation of PCR products. BioTechniques 12(1):28.

Lundeberg, J., J. Wahlberg, and M. Uhlén. (1991). Rapid colorimeter quantification of PCR-Amplified DNA. BioTechniques 10(1):68-74.

Mead, D. A., N. K. Pey, C. Herrnstadt, R. A. Marcil, and L. M. Smith. (1991). A universal method for the direct cloning of PCR amplified nucleic acid. BioTechnology 9:657-663.

Monaco, A. P. and Z. Larin. (1994). YACs, BACs, PACs, and MACs: Artificial chromosomes as research tools. Trends in Biotechnology 12:280-286.

Ochman, H., F. J. Ayala, and D. L. Hartl. (1993). Use of polymerase chain reaction to amplify segments outside boundaries of known sequences. Methods of Enzymology. 218:309-321.

Olsen, D. B., G. Wunderlich, A. Uy, and F. Eckstein. (1993). Direct sequencing of Polymerase Chain Reaction products. Methods of Enyzmology. 218:79-93.

Penner, G. A., A. Bush, R. P. Wise, W. Kim, L. Domier, K. Kasha, A. Laroche, G. Scales, S. J. Molnaer, and G. Fedak. (1993). Reproducibilty of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR methods and applications, Cold Spring Harbor Laboratories. 2:341-345.

Powell, W., M. Morgante, C. Andre, M. Hanafrey, J. Vogel, S. Tingey, and A. Rafalski. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2:225-238.

Prince, A. M. and L. Andrus. (1992). PCR: How to kill unwanted DNA. BioTechniques 12(3):358-359.

Ragot, M. and D. A. Hoisington. (1993). Molecular markers for plant breeding: Comparison of RFLP and RAPD genotyping costs. Theor. Appl. Genet. 86:975-984.

Rychlik, W. (1995). Priming efficiency in PCR. BioTechniques 18(1):84-90.

Sankar, G. and S. S. Sommer (1993). Removal of DNA contamination in polymerase chain reaction reagents by ultraviolet irradiation. Methods in Enzymology. 218:381-388.

Schwarz, H. and J. Lindsay. (1992). A rapid, inexpensive method for eluting dna from agarose or acrylamide gel slices without using toxic or chaotropic materials. Biotechniques 13(2):205-206.

Schweder, M. E., R. G. Shatters, Jr., S.H. West, and R. L. Smith. (1995). Effect of transition interval between melting and annealing temperatures on RAPD analysis. BioTechniques 19(1):38-42.

Shahjahan, R. M., K. J. Hughes, R. A. Leopold, and J. D. DeVault. (1995). Lower incubation temperature increases yield of insect genomic DNA isolated by the CTAB method. BioTechniques 19(3):332-334.

Shuldiner, A. R., L. A. Scott, and J. Roth. (1990). PCR-induced (ligase-free) subcloning: A rapid reliable method to subclone polymerase chain reaction (PCR). Nucleic Acids Research 18(7):1920.

Skolnick, M. H. and R. B. Wallace. (1988). Simultaneous analysis of multiple polymorphic loci using amplified sequence polymorphisms (ASPs). Genomics 2:273-279.

Skroch, P. and J. Nienhuis. (1995). Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor Appl Genet 91:1086-1091.

Steiner, J. J., C. J. Poklemba, R. G. Fjellstrom, and L.F.Elliott. (1995). A rapid one-tube genomic DNA extraction process for PCR and RAPD analysis. Nucleic Acids Research 23(13):2569-2570.

Thomson, D. and R. Henry. (1995). Single-step protocol for preparation of plant tissue for analysis by PCR. BioTechniques 19(3):394-400.

W., R. Hot prospect for New Gene Amplifier. Science 254:1292.

Williams, J. G. K., M. K. Hanafey, J. A. Rafalski, and S. V. Tingley. (1993). Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzymology, Academic Press. 218:704.

Wittmer, C. T. and D. J. Garling. (1991). Rapid cycle DNA amplification: Time and Temperature Optimization. BioTechniques 10(1):76-83.

ACKNOWLEDGEMENTS

I would like to thank all of the lab personnel past and present for their help on my thesis. I would like to especially like to thank my advisor and supervisor, Dr. Roger Wise, for giving me this opportunity to achieve this goal. I would like to thank Drs. Rich DeScenzo, Arla Bush, and Shaun Morroll for their help in editing and answering my questions. I would like to thank my POS committee for waiting all these years for me to finish this degree program and allowing me to be "special". Finally, I would like to thank my family for waiting all these years for me to finish this degree program. Now our lives can go back to normal.